

Subgeneric diversity of *Brasilonema* (Cyanobacteria, Scytonemataceae)

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ABSTRACT – (Subgeneric diversity of *Brasilonema* (Cyanobacteria, Scytonemataceae)). The recently described scytonematoid cyanobacterial genus *Brasilonema* is known mainly from tropical and subtropical rain forests (Mata Atlântica) of southeastern Brazil, where it occurs in aerophytic wooden, stony and iron substrates. This genus was defined according to both molecular and morphological criteria. The type species *B. bromeliae* was described from the specialized habitat: it grows in phytothelms, epiphytic on both living and died leaves within the rosettes of large bromeliad plants slightly above or in the zone of the water level. The genus *Brasilonema* is evidently widely distributed in coastal forests of São Paulo State, where it occurs also in remarkable diversity. According to our results, this genus currently comprises seven taxa, which are distinct by different morphology and ecological characteristics.

Key words - *Brasilonema*, Brazil, new species, subaerophytic habitats

RESUMO – (Diversidade subgenérica de *Brasilonema* (Cyanobacteria, Scytonemataceae)). O gênero scytonematóide de Cyanobacteria, *Brasilonema*, foi descrito recentemente e é conhecido principalmente de florestas tropicais e subtropicais (Mata Atlântica) da região sudeste do Brasil. Ocorre sobre substratos aerofíticos como madeira, pedra e ferro e foi definido de acordo com critérios moleculares e morfológicos. A espécie-tipo, *B. bromeliae*, foi coletada em um habitat muito específico: epífita em folhas de bromélias, dentro das rosetas, próximo do nível da água, ou ainda em folhas secas destas plantas. O gênero *Brasilonema* é amplamente distribuído nas florestas costeiras do Estado de São Paulo, onde ocorre em alta diversidade morfológica. De acordo com nossos resultados, este gênero atualmente compreende sete táxons distintos com base nas diferenças morfológicas e características ecológicas.

Palavras chave - *Brasilonema*, Brasil, espécies novas, habitats aerofíticos

Introduction

The diversity of cyanobacteria in tropical regions, particularly in extreme habitats with specialised environmental conditions, is still insufficiently known. In subaerophytic sites of humid tropical and subtropical forests of southeastern Brazil (Mata Atlântica) it was found a special cyanobacterial type, phenotypically similar to the genus *Scytonema*, but growing in characteristic erect fascicles and with rare false branching. After combined molecular and phenotype evaluation, this type distinct and different from *Scytonema* was recognised as a separated generic entity. It was described under the name *Brasilonema* according to both Bacteriological and Botanical nomenclatoric rules by Fiore *et al.* (2007).

The type species of *Brasilonema*, *B. bromeliae*, has an unusual ecology. It grows in phytothelms, epiphytic on

both living and died leaves inside of the rosettes of large epiphytic species of bromeliads (from the genera *Vriesea*, *Aechmea*, *Wittrockia*, *Neoregelia* and others), on the limit of the water level. However, the *Brasilonema*-types also occur on different subaerophytic habitats mainly in humid forests along the coastal mountains in the São Paulo State. They were found on bark of trees and various wetted wooden, stony and iron substrates. The various populations differ by ecology and by morphological deviations, and they represent an interesting cyanobacterial diversified cluster in these tropical and subtropical regions. The various eco and morphotypes should be described as different morphospecies in agreement with the accepted criteria of cyanobacterial taxonomy.

The morphological deviations of these morphotypes were also the reason why Komárek (2003) described one of the species, which belongs evidently to this taxonomic unit, as a member of the similar heterocytous genus *Camptylonemopsis* (*C. sennae* Komárek). The similarity to *Camptylonemopsis* is in the fasciculation of creeping filaments on the substrate and sparse false branching. However, the filaments of *Camptylonemopsis* are shorter, their creeping by the central parts of filaments is obligatory, and the isopolar filaments have a morphologically middle part distinct from the erect

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terminal ends (variation in cell form). The akinetes were described in the type species *C. lahorensis* (Ghose) Desikachary (cf. Desikachary 1959, Komárek 2003). The molecular analyses proved the close relationship of *C. sennae* to the genus *Brasilonema* (Aguiar *et al.* 2008).

Another species of this morphotype was characterised by Bourrelly (*in* Bourrelly & Manguin 1952) from Guadeloupe, originally described as *Tolypothrix roberti-lamyi* Bourrelly, and later recombined by Komárek (1989) into the genus *Schmidleinema*. This species has similar initial stages as *Schmidleinema*, but the fasciculation of filaments, absence of true branching and very rare tolypotrichoid false branching correspond rather to the new Brazilian type. It is different from the diagnoses of *Schmidleinema*, *Tolypothrix*, *Scytonema* and *Camptylonemopsis*. According to morphological similarity, the identification of *Brasilonema* populations described under these different generic names must be considered only as preliminary and nomenclatorically invalid in the light of the recent results.

After definition of the genus *Brasilonema*, seven taxa were recognised. The 16S rRNA gene phylogenetic analysis and the phenotype descriptions are presented in this article.

Material and methods

Twelve samples of the genus *Brasilonema* collected in São Paulo State, Southeastern Brazil, were used for description of its diversity. Among these samples, eight were collected in different localities from the humid Atlantic Forest (Cardoso Island, Juréia, Paranapiacaba and Campos do Jordão), three in the Botanical Garden of São Paulo, and one in Águas de São Pedro Village. Data of *B. octagenarum* Aguiar *et al.* were taken from Aguiar *et al.* (2008). The samples were collected scraping substrates (stone, concrete, iron and wood) using a spatula. Fresh and preserved materials (dry and in formaldehyde solution) were studied under Zeiss Axioplan-2 optical microscope and photographed with coupled camera. The taxa identification was done based on at least 20 specimens of each sample. The reference strain SPC 951 of the type species *B. bromeliae* and other strains are cultivated in liquid BG-11 medium (Allen 1968) without inorganic nitrogen source.

The almost complete 16S rRNA gene from the genomic DNA of the *B. terrestre* CENA116 was amplified by PCR using the oligonucleotide primers 27F1 and 1494Rc (Neilan *et al.* 1997) corresponding to the *Escherichia coli* 16S rRNA gene positions 27 to 1494. The PCR amplification and sequencing were carried out as described previously (Fiore *et al.* 2007). The 16S rRNA sequences of *B. bromeliae* SPC951, *B. sennae* CENA114 and *B. octagenarum* strains UFV-E1 and UFV-OR1 were obtained in earlier study (Fiore *et al.* 2007,

Aguiar *et al.* 2008). The *B. terrestre* CENA116 nucleotide sequence obtained in this study has been deposited in GenBank under accession number EF490447. The 16S rRNA gene sequence obtained in this study and reference sequences retrieved from GenBank were aligned, refined and used to generate a phylogenetic tree. The tree was reconstructed using the Neighbor-Joining (NJ) method implemented by the MEGA version 4.0 program package (Tamura *et al.* 2007) using the Kimura 2-parameter sequence evolution model. The robustness of the tree was estimated by bootstrap percentages using 1,000 replications.

Results and discussion

Six different taxa were recognized within the genus *Brasilonema* on the basis of Brazilian natural populations and their cultures. One of them (*B. terrestre* Sant'Anna & Komárek) is presently described also on molecular base (figure 1, table 1). The seventh taxa, *B. roberti-lamyi* Sant'Anna & Komárek, is a new combination based on *Tolypothrix roberti-lamyi* Bourrelly from Guadeloupe.

Brasilonema bromeliae Fiore *et al.*, J. Phycol. 43:789-798. 2007.

Figure 2

Thallus macroscopic, velvet-like, blackish-green to blackish-violet, densely fasciculated. Filaments 15-17 µm wide, firstly more or less creeping on the substrate, slightly irregularly coiled, cylindrical, later joined in dense erect fascicles. Sheaths firm, cylindrical, slightly lamellated, colorless up to yellowish-brown. Trichomes 14.5-16.8 µm wide, not or very slightly constricted at cross-walls, not attenuated. Cells as long as wide to shorter than wide; cell content slightly granular, greyish-blue, blue-green, olive-green or brownish-violet; vacuole-like structures sometimes in cells of apical (young) parts. Heterocytes solitary, rounded or short cylindrical, 14-19 µm long, 15-16.8 µm wide.

Habitat: in the inner side of bromeliads leaves and on wooden substrates in greenhouse, São Paulo, Brazil.

Brasilonema epidendron Sant'Anna et Komárek, *sp. nov.*

Typus: Brasília, Provincia São Paulo, Municipium Peruíbe, Juréia (locus classicus), in substrata lignosa, in sylvis humidis, Mata Atlântica dictis, IX-2002, C.L. Sant'Anna, s.n. (BRNM/HY 1333) (Holotypus BRNM/HY).

Figures 3-7, 9

Thallus cum filamentis ± paralleliter irregulariterque fasciculatis, erectis, stratum atro-viride ad nigrescente formans; fasciculi erecti ad 3 mm alti. Filamenta

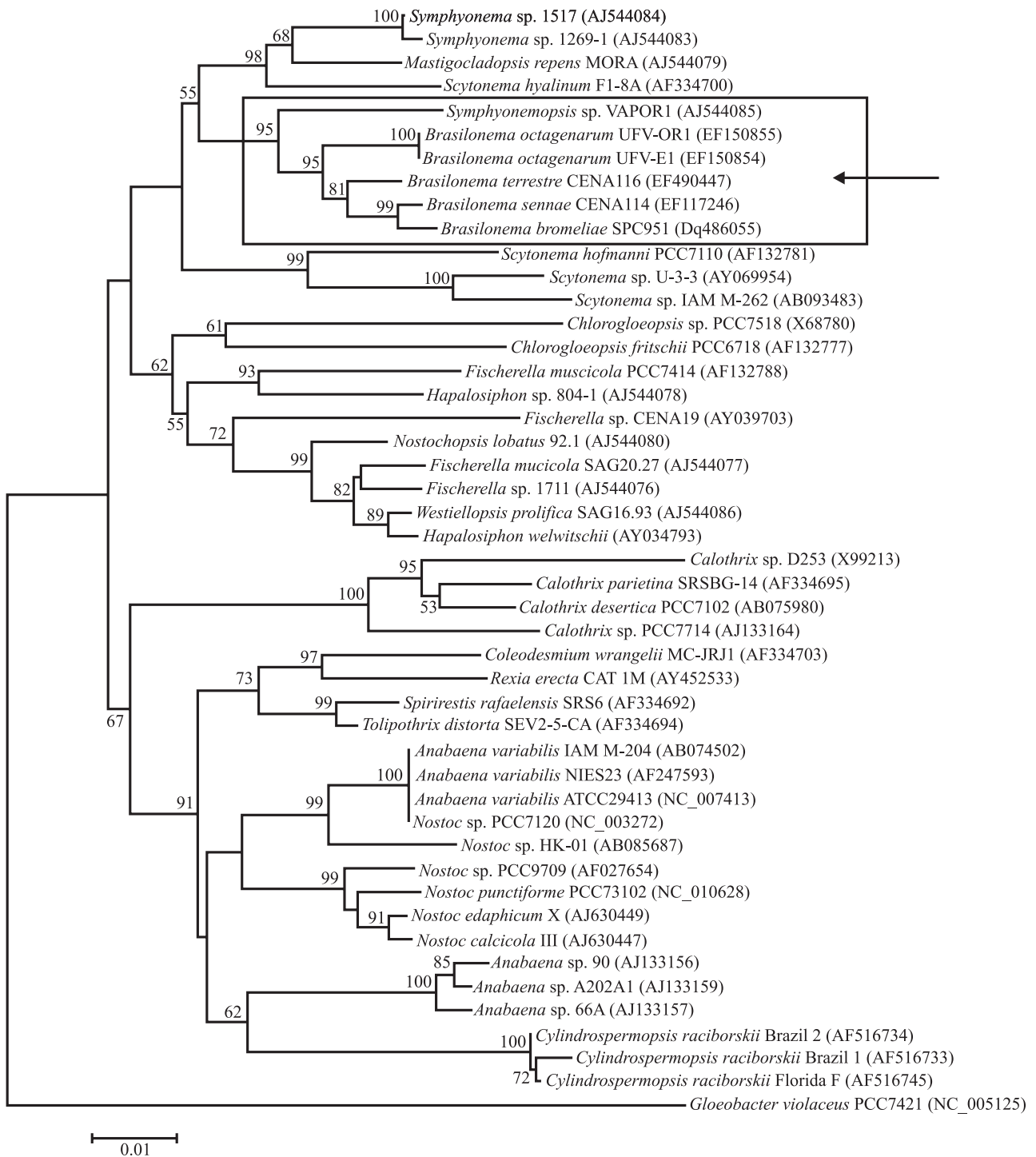


Figure 1. Neighbour-joining tree with phylogenetic position of the *Brasilonema* strains (including type strain *B. bromeliae* SPC951), and related cyanobacteria based on 16S rRNA sequences (878 bp). Numbers near nodes indicated bootstrap values over 50% (1000 replicates).

cylindrica, (7)10.9-12(14) μ m lata, dense fasciculata. *Trichoma cylindrica*, (5.5)8.2-10(11) μ m lata, ad dissepimenta not constricta, sed in hormogonia constricta. Vaginae tenues, firmae, superficie leves, sine colore. Cellulae plus minusve isodiametricae,

cylindricae, sed ad apicibus valde brevius, contentu vivide aeruginosae. Hormogonia cum cellulis brevis, granulatis, plus minusve 5,5 μ m lata, ad dissepimenta leviter constricta. Heterocystae solitariae, barriliformes vel cylindricae, (7)8-10(11.5) μ m longis, 7-9 μ m lata.

Table 1. 16S rRNA gene sequence similarity of the *Brasilonema* strains. Identity [%].

Strain	Origin	2	3	4	5	6
1. <i>B. bromeliae</i> SPC951	Epiphytic on inner part of Bromelias leaves, above water level; Botanical Garden, São Paulo	99.3	97.2	96.5	96.7	96.1
2. <i>B. sennae</i> CENA114	Subaerophytic, on wet iron tube; near Paranapiacaba, São Paulo	–	97.2	97.1	97.2	96.0
3. <i>B. terrestre</i> CENA116	Subaerophytic, on periodically wet concrete substrate; Cardoso Island		–	97.6	97.7	96.3
4. <i>B. octagenarum</i> UFV-E1	Epiphytic on <i>Eucalyptus</i> surface leaves, Timóteo, Minas Gerais			–	99.9	96.0
5. <i>B. octagenarum</i> UFV-OR1	Epiphytic on orchid surface leaves, Venda Nova do Imigrante, Espírito Santo				–	96.0
6. <i>Symphyonemopsis</i> VAPOR1	Cave of El Vapor, karstic region of Castillo hill, near Alhama, Murcia, SE Spain					–

Thallus macroscopic, dark green to blackish, forming irregular erect fascicles, up to 3 mm high. Filaments (7)10.9-12(14) μm wide, parallel. Sheaths thin, firm, colourless. Trichomes (5.5)8.2-10(11) μm wide not constricted at cross walls, not attenuated. Cells so long as wide or cylindrical, very short in apical part; cell content bright blue-green. Hormogonia with short cells slightly constricted at cross walls, granulated. Heterocytes solitary, barrel-shaped to cylindrical, (7)8-10(11.5) μm long, 7-9 μm wide.

Habitat: Subaerophytic, corticolous or on old wooden substrates in Atlantic Forest.

Localities: Brazil, SP, Juréia, coordinates 20°17'85" S and 46°22'17" W (locus classicus) and Paranapiacaba.

Brasilonema octagenarum Aguiar *et al.*, J. Phycol. 44: 1322-1334. 2008

Figure 8

Thallus macroscopic, forming mats or irregular creeping fascicles dirty green, brownish or blackish-green. Filaments 9.8-18.5 μm wide, cylindrical. Sheaths thin, firm, cylindrical, sometimes slightly lamellated, colorless. Trichomes 9.5-18.4 μm wide, not constricted at cross-walls, not attenuated. Cells 1.5-13.3 μm long; cell content granular, olive-green, brownish or rarely violet; vacuole-like structures sometimes in cells of apical and differentiate parts. Heterocytes solitary, terminal or intercalary, discoid or more or less cylindrical, 5.4-15.6 μm long, 10-17 μm wide. Hormogonia with short, more granulated cells, 4.8-12.6 μm wide. Hormocysts isodiametric, thick wall, 6.5-10.2 μm long, 6.1-10.6 μm wide.

Habitat: epiphytic on leaves, stem and bud of *Eucalyptus grandis* Hill ex Maiden, Minas Gerais State, Brazil.

Brasilonema ornatum Sant'Anna et Komárek, *sp. nov.*

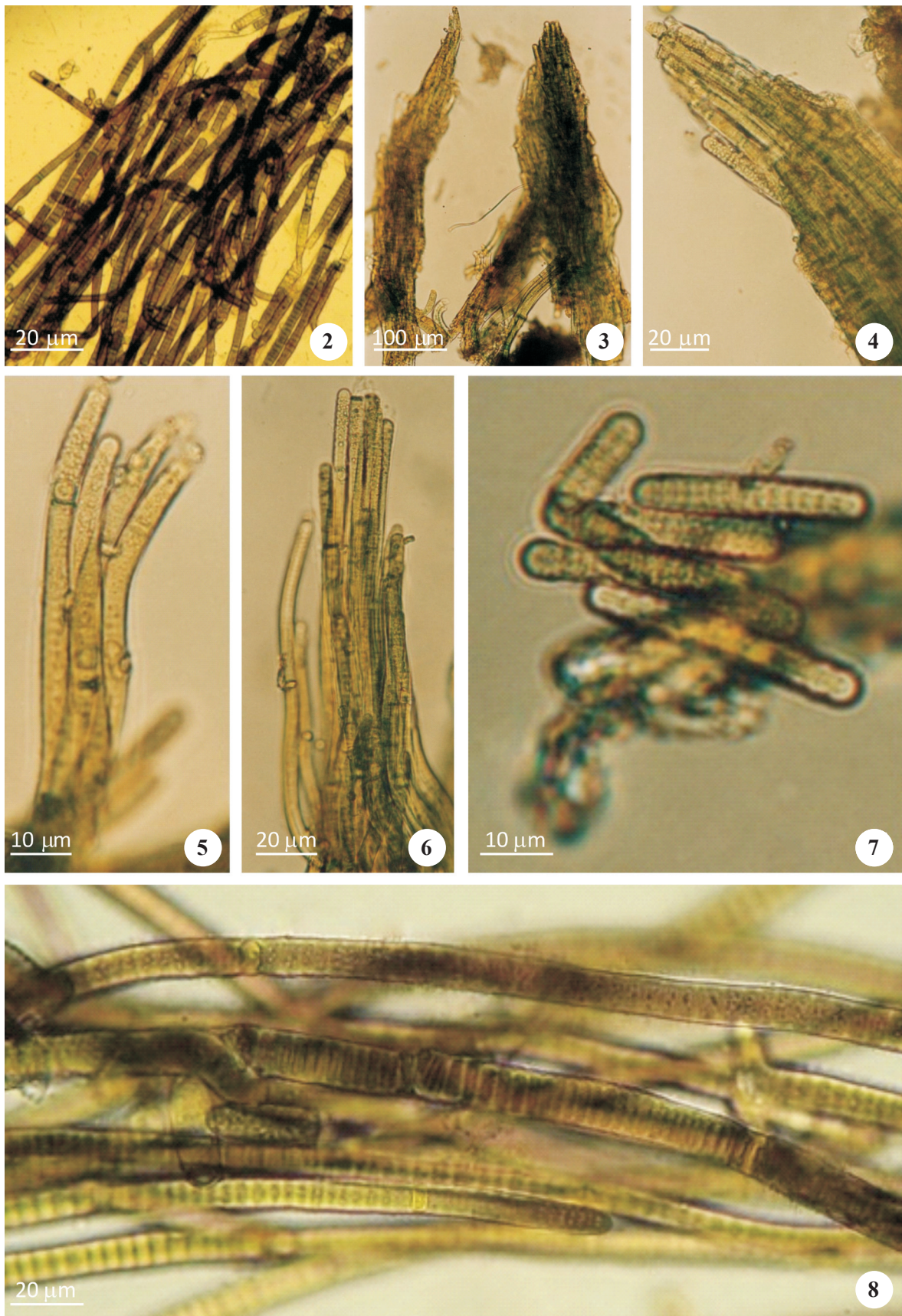
Typus: Brasília, Provincia São Paulo, Municipium Campos do Jordão, in sylvis montanosis prope Campos do Jordão (locus classicus), subaerophytice, corticolae lignicolaeque, seape inter lichenes muscisque, XI-2002, *M.T.P. Azevedo, J. Komárek et C.L. Sant'Anna, s.n.* (icona typica figura nostra 10A-D)
Figure 10A-D

Thallus cum filametis irregulariter fasciculatis, superficie substrata lignosa repens, interdum stratum leve, griseo-viride formans. Filamenta cylindrica, 20-23 μm lata. Trichoma cylindrica, 17-18 μm lata, ad apices non attenuata, ad dissepimenta distincte constricta. Vaginae crassae, lamellosae, superficie regulariter ornamentosae (areolatae), sine colore. Cellulae semper brevior quam latae, contentu plus minusve homogoneo, atro-viridi, breve barriliformes. Heterocystae rotundo, 17-18 μm longis, 3-6 μm lata.

Thallus macroscopic, greyish green, forming irregular fine mats with creeping, partly fasciculated filaments. Filaments 20-23 μm wide. Sheaths thick, lamellated, regularly ornamented, colourless. Trichomes 17-18 μm wide, constricted, not attenuated. Cells short barrel shaped; cell content dark blue-green. Heterocytes rounded, 17-18 μm long, 3-6 μm wide.

Habitat: Subaerophytic, on bark of trees among mosses and lichens.

Locality: Brazil, SP, Campos do Jordão (Horto Florestal), coordinates 22°45'19" S and 45°35'25" W (locus classicus).



Figures 2-8. *Brasilonema* taxa. 2. *Brasilonema bromeliae*. 3-7. *Brasilonema epidendron*. 8. *Brasilonema octogenarum*.

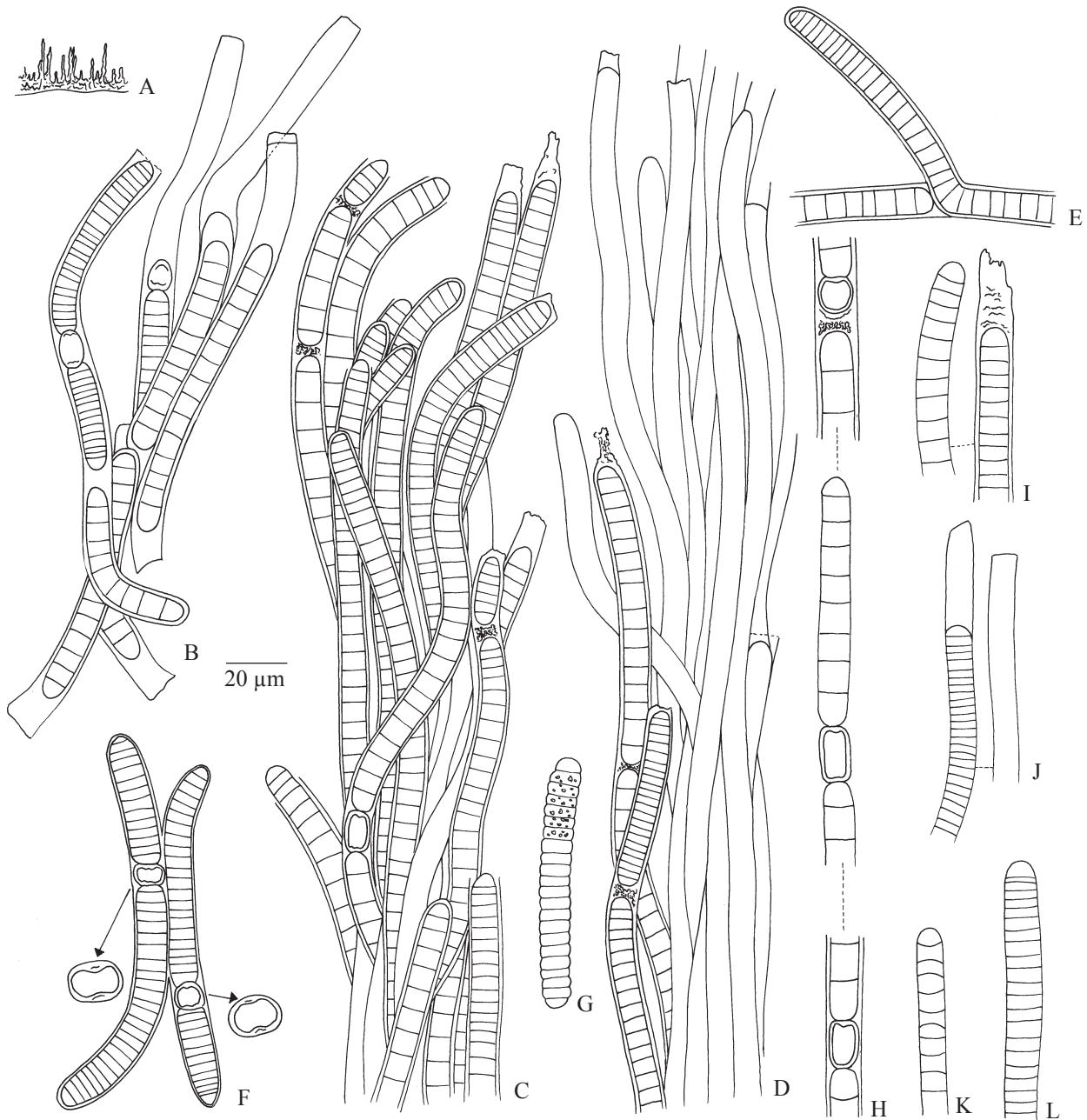


Figure 9. *Brasilonema epidendron*. A. Scheme of macroscopic thallus. B-D. Fascicles of filaments. E. False tolypotrichoid branching. F. Details of hormogones with intercalary heterocytes (arrows indicate scheme of heterocytes). G. Hormogonium. H. Part of trichomes with heterocytes. I-L. Ends of filaments and trichomes.

Brasilonema roberti-lamyi (Bourrelly) Sant'Anna et Komárek, *comb. nov.*

Basionym: *Tolyptothrix roberti-lamyi* Bourrelly in Bourrelly & Manguin, *Algues d'eau douce Guadeloupe*. 151. 1952.

Figure 11

Thallus macroscopic, forming erect fascicles of \pm parallel arranged filaments. Filaments 12-18 μm .

Sheaths firm, thick, colourless, finally lamellated. Cells so long as wide or slightly longer or shorter than wide, apical cells barrel shaped to rounded; cell content dark blue-green. Heterocytes discoid to cylindrical. Reproduction by hormogonia with lamellated sheaths (according to Bourrelly & Manguin 1952).

Habitat: Aerophytic, Guadeloupe.

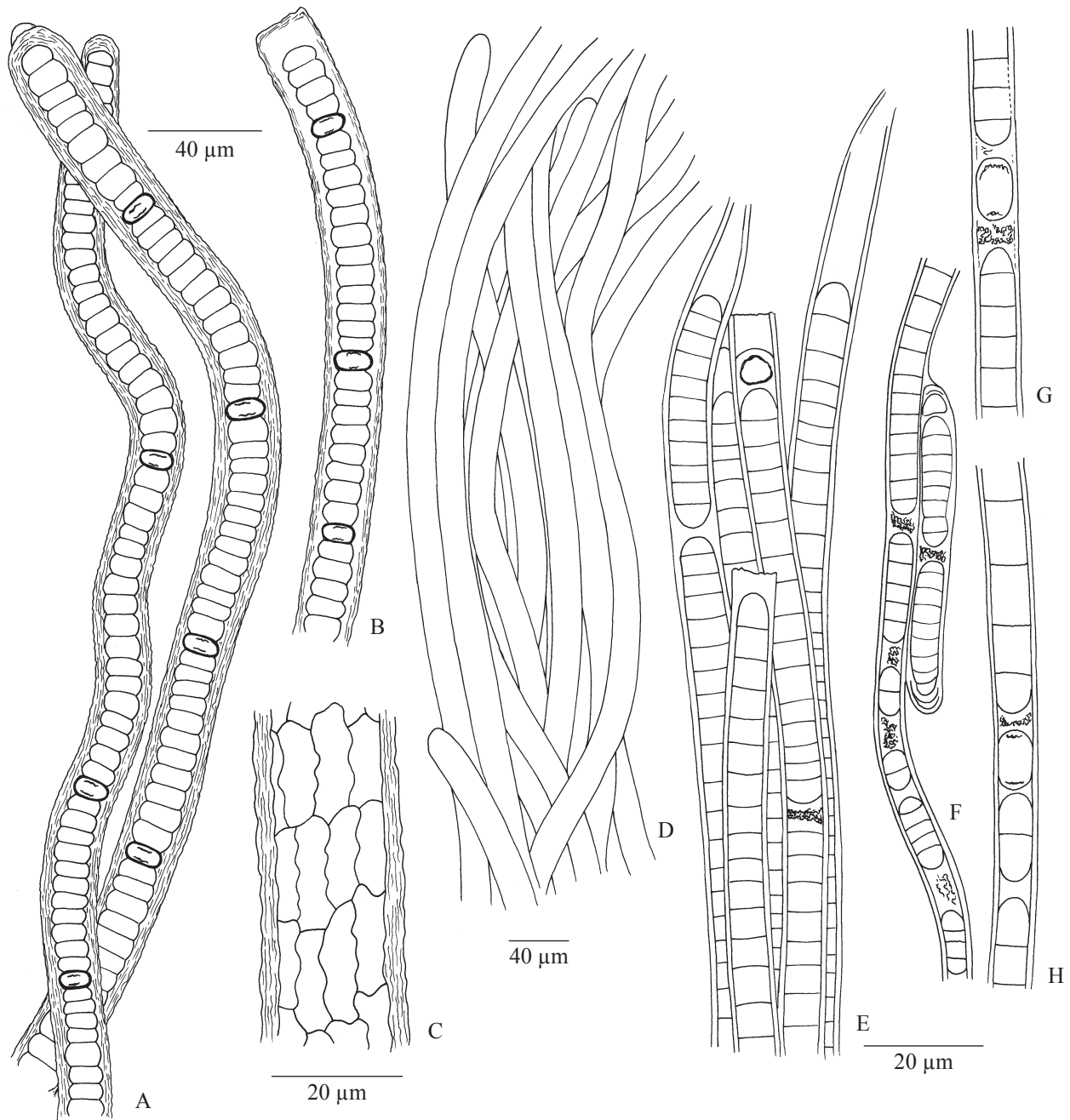


Figure 10. A-D. *Brasionema ornatum*. A-B. Filaments. C. Detail of sheath surface. D. Fasciculation of trichomes. E-H. *Brasionema sennae*. E. Details of fascicle. F. Trichomes with hormogonia. G-H. Details of filaments with heterocytes. (E-H from Komárek 2003, as *Camptylonemopsis sennae*).

Brasionema sennae (Komárek) Sant'Anna et Komárek, *comb. nov.*

Basionym: *Camptylonemopsis sennae* Komárek, *Preslia* 75: 225. 2003

Figure 10E-H

Thallus macroscopic, woolly mats, brownish or blackish-green, regular parallel fascicles. Filaments

10-20 µm wide, slightly wavy or coiled, with sparsely tolypotrichoid or scytonematoid branching at free ends. Sheaths thin, firm, later thick, colourless when young, old sheaths lamellated and yellowish-brown. Trichomes 6-12.5 µm wide, not constricted at cross walls, not attenuated. Cells cylindrical, variable in length, particularly in hormogonia and young tri-chomes (from the length 2.2 µm), in old trichomes up to 1.6 longer than

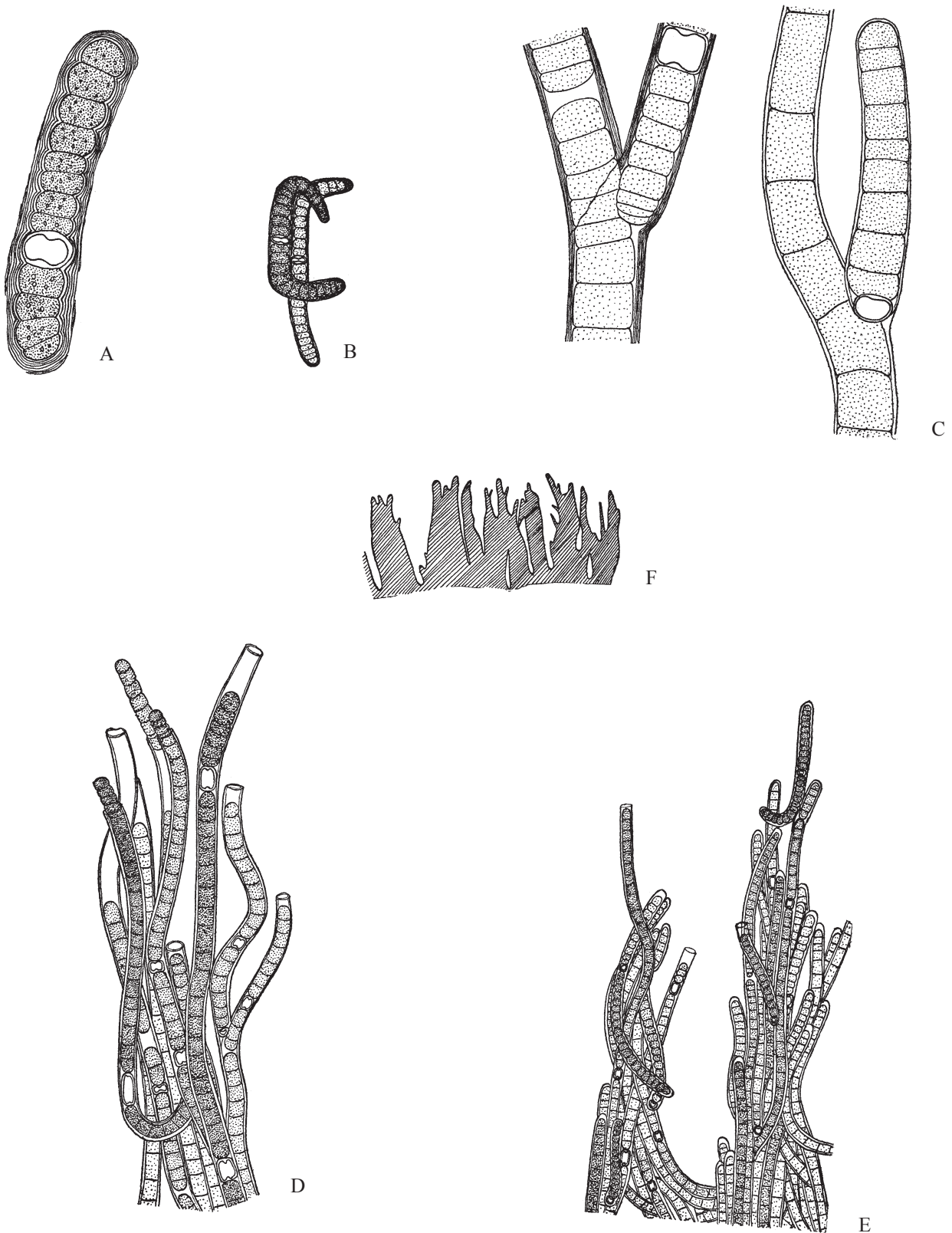


Figure 11. *Brasilonema roberti-lamyi*. A. Hormogonium. B. Germinating hormogonia. C. Tolypotrichoid branching. D-E. Fasciculation of filaments. F. Macroscopic colonies. (From Bourrelly & Manguin 1952, A e C = 840x; B, D e E = 225x; F = 26x).

wide; cell content blue-green or olive-green. Heterocytes solitary, cylindrical, 6.8-15.4 μm long, 10.2-11.2 μm wide.

Habitat: From wetted subaerophytic stony and iron substrates near springs, several localities are known from the vicinity of Paranapiacaba, SP, Brazil.

Brasilonema terrestre Sant'Anna et Komárek, sp. nov.

Typus: Brasília, Provincia São Paulo, Municipium Cananeaia, Insula Cardoso, subaerophytice in substrata saxosa periodice humida, VI-2003, C.L. Sant'Anna, s.n. (SP 399777) (Holotypus SP)

Figure 12

Thallus cum filamentis irregulariter in fasciculis consociatis, plus minusve erectis, stratum atrocyaneum macroscopicum formans. Filamenta cylindrica, 12-17 μm lata, dense fasciculata. Trichoma cylindrica, 9-15 μm lata, ad apices leviter attenuata, ad dissepimenta non constricta. Vaginae tenues, firmae, superficie leve granulatae vel lineatae, sine colore. Cellulae plus minusve isodiametricae vel brevior cum latae, praecipue ad apices, contentu griseo-viridi vel atrocyaneae. Heterocystae solitariae, barriliformes vel cylindricae, 6-17 μm longis, 13-14 μm latae.

Thallus macroscopic, dirty-green, forming mats or irregular fasciculated filaments. Filaments 12-17 μm . Sheaths thin, firm, yellow-brown, slightly ornamented at the surface (granular or finely lineated). Trichomes 9-15 μm wide, not constricted, not attenuated. Cells as long as wide, shorter to the ends (up to 1/3 of their width); cell content greyish-green or dirty blue-green. Heterocytes barrel-shaped to cylindrical, 6-17 μm long, 13-14 μm wide.

Habitat: Subaerophytic on concrete.

Localities: Brazil, SP, Cardoso Island, coordinates 25°07'59" S and 47°58' 19" W (locus classicus) and Águas de São Pedro Village.

During the study of subaerophytic cyanobacterial microflora of Atlantic Forest, São Paulo State, SE Brazil, it was recognised a special genus *Brasilonema* from the family Scytonemataceae. Its separate position from the closest genera (*Scytonema*, *Mastigocladopsis*, and *Symphyonema*) was proved by molecular sequencing as shown in figure 1.

Among the seven *Brasilonema* taxa, *B. bromeliae*, *B. sennae* and *B. octagenarum* were also characterized based on molecular data (Fiore *et al.* 2007, Aguiar *et al.* 2008) and *Brasilonema terrestre* is presently described. The other morphospecies (*B. epidendron*, *B. ornatum* and *B. robert-lamyi*) differ from all *Brasilonema* species

in dimensions (width of filaments and trichomes), coloration of cells (phycobilin pigment ratio) and sheaths, and ecology (type of substrates) and are presently described. The regular ornamentation of the sheath surface in *B. ornatum* is specific and unique in cyanobacteria. It was firstly described by Sant'Anna *et al.* (1983) in an atypical population of *Scytonema stuposum* (Kützinger) Bornet, also from a corticolous habitat (mangrove trees) in the State of São Paulo. The authors mentioned the possibility that the ornamentation could be resultant from a special type of lichenization. This phenomenon is surely interesting and should be explained in future. No hyphae were recognized in the populations presently examined, and the origin (formation) of this sheath ornamentation still remains unclear. Finally, *Tolypothrix roberti-lamyi*, described from aerophytic habitats in Guadeloupe, belongs evidently also to this genus according to morphological markers, and was taxonomically re-combined. The comparison of phenotype characters of all species is given in table 2.

According to molecular evaluations (Fiore *et al.* 2007, Aguiar *et al.* 2008), *Brasilonema* represents a concise cluster distinctly separated from other groups. The inner diversity is uniform, and the separation in various species was supported according to the formal bacteriological criteria (Wayne *et al.* 1987, Stackebrand & Goebel 1994) in four species (table 1). The similarity between different morphotypes is usually higher than 96%. However, the different ecotypes are distinguishable according to morphological criteria (table 2) and the occurrence of different stable morphotypes repeats in different localities. It is evidently the same type of diversity, like in other uniform cyanobacterial genera (*Microcystis*, *Planktothrix*), where the method of 16S rRNA sequencing cannot separate clearly different stable types. They are distinguished by morphological, ecological, and biochemical characters.

The similarities of 16S rRNA gene sequences among five *Brasilonema* strains, which characterize four species, are included in table 1. These sequences showed similarities lower than 94% with sequences from *Scytonema* strains, the closest morphotype at the generic level. However, at the genotype level, *Brasilonema* strains were close to *Symphyonemopsis* VAPOR1, which has a true branching morphotype. The phylogenetic analysis also grouped the *Brasilonema* strains with *Symphyonemopsis* VAPOR1 (figure 1). The morphology of the strain designated "*Symphyonemopsis* VAPOR 1" isolated from Spain is unknown and

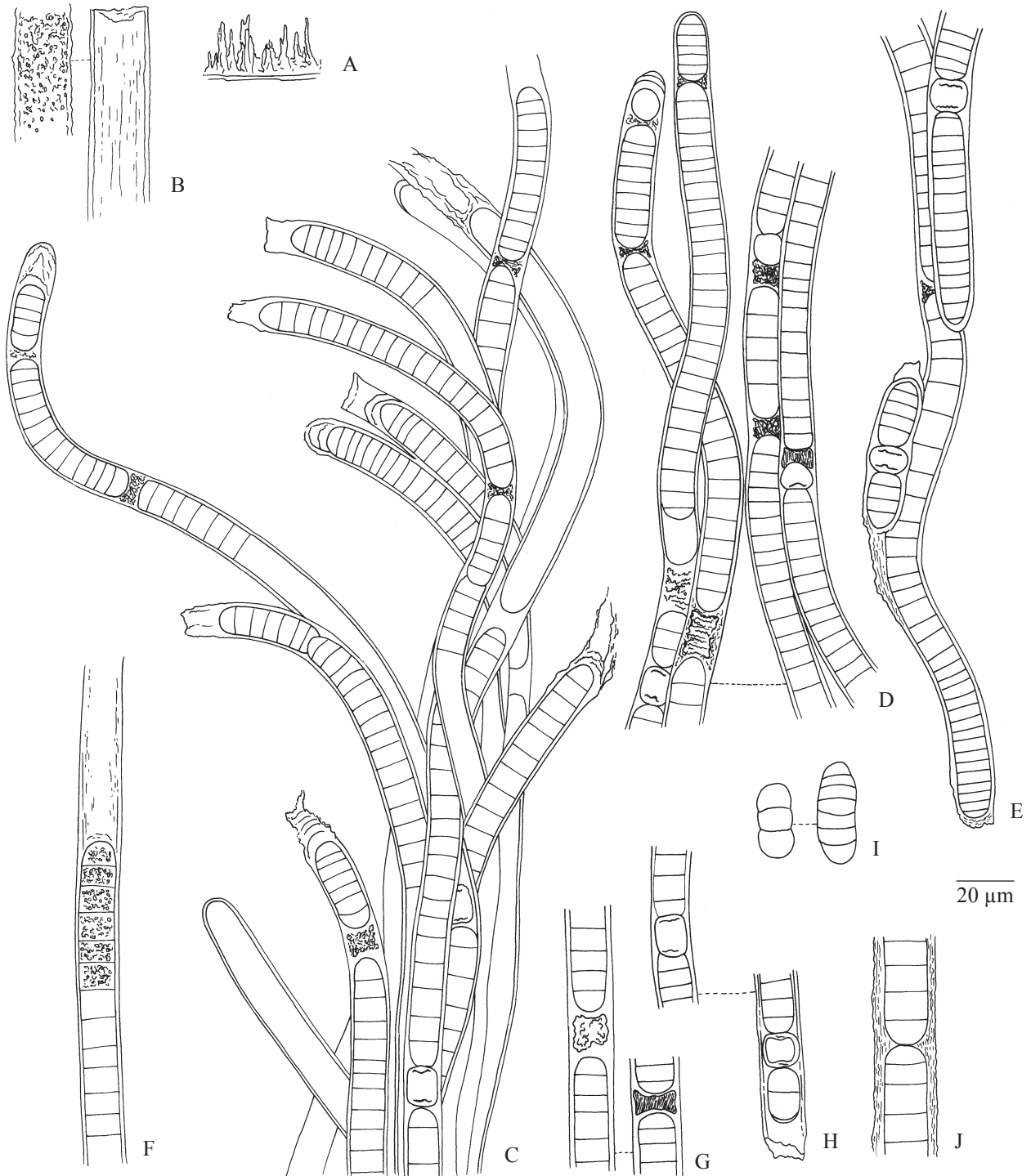


Figure 12. *Brasilonema terrestre*. A. Scheme of macroscopic thallus. B. Scheme of morphology of structured sheaths. C-D. Fasciculated filaments. E. False branching. F. End of a filament. G-J. Fragmentation of trichomes in hormogonia. H. Part of trichomes with heterocytes. I. Hormogonia.

must be explained in future. Thus, further sampling of *Symphyonemopsis* taxa is required before the phylogenetic position of this group can be solved. However, the relation of this genus with *Brasilonema*

is improbable. The molecular data obtained in this study corroborate earlier results (Fiore *et al.* 2007, Aguiar *et al.* 2008) and reinforce the separate position of *Brasilonema* strains at the generic level.

Table 2. Phenotypic review of the genus *Brasilonema*.

	<i>B. bromeliae</i>	<i>B. epidendron</i>	<i>B. octagenarum</i>	<i>B. ornatum</i>	<i>B. roberti-lamyi</i>	<i>B. sennae</i>	<i>B. terrestre</i>
Width of filaments (µm)	15-17	(7)10.9-12(14)	9.8-18.5	20-23	12-18	10-20	12-17
Width of trichomes (µm)	14.4-16.8	(5.5)-8.2-10(11)	9.5-18.4	17-18		6-12.5	9-15
Morphology of sheaths	Thin, firm	Thin, firm	Thin, firm, later slightly lamellated	Thick, lamellated, ornamented	Thick, finely lamellated	Thin, firm, later lamellated	Thin, firm, slightly ornamented
Colour of sheaths	Colourless, rarely to yellowish-brown	Colourless	Colourless	Colourless	Colourless	Rarely colourless partly yellow-brown	Colourless to yellow-brown
Colour of cells	Greyish blue or brownish, olive-green, or violet	Bright blue-green	Brownish, olive-green, rarely violet	Dark blue-green	–	Blue-green or olive-green	Greyish-green or blue-green
Form of thallus	Free fascicles	Irregular erect fascicles	Mats to irregular fascicles, creeping fascicles.	Creeping fascicles	Mats to erect fascicles	Regular erect fascicles	Mats to irregular erect fascicles
Colour of thallus	Blackish-green to blackish violet	Dark green to black (dried)	Dirty green, brownish or blackish-green	Greyish-green	–	Dirty green, brownish, or blackish-green	Dirty green
Heterocytes (µm)	Rounded or short cylindrical 4-19 × 15-16.8	Barrel-shaped to cylindrical (7)8-10(11.5) × 7-9	Discoid or ± cylindrical, 5.4-15.6 × 10-17.6	Discoid 3-6 × 17-18	Discoid to cylindrical	Cylindrical 6.8-15.4 × 10.2-11.2	Barrel-shaped to cylindrical 6-17 × 13-14
Ecology	Subaerophytic, epiphytic on living and dead leaves of bromelias (inside of leaf rosettes)	Subaerophytic, corticolous, on old wooden substrates in rainy forests	Epiphytic on leaves, stem and buds of <i>Eucalyptus grandis</i>	Subaerophytic, on bark of old trees among mosses and lichens	“Aerophytic”	Subaerophytic, edge of springs on wet wooden stony and iron substrates	Subaerophytic, on periodically wetted stony substrates
Distribution	Brazil (SP), São Paulo City, Paranapiacaba village	Brazil (SP), Paranapiacaba village, Parque Estadual Juréia-Itatins	Brazil (MG), Timoteo	Brazil (SP), Campos do Jordão	Guadeloupe	Brazil (SP), Paranapiacaba village	Brazil (SP), São Pedro village, Cardoso Island

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References

- AGUIAR, R., FIORE, M.F., FRANCO, M.W., VENTRELLA, M.C., LORENZI, A.S., VANETTI, C.A. & ALFENAS, A.C. 2008. A novel epiphytic cyanobacterial species from the genus *Brasilonema* causing damage to *Eucalyptus* leaves. *Journal of Phycology* 44:1322-1334.
- ALLEN, M.B. 1968. Simple conditions for growth of unicellular blue-green algae on plates. *Journal of Phycology* 4:1-4.
- BOURRELLY, P. & MANGUIN, E. 1952. Algues d'eau douce de la Guadeloupe et dépendances recueillies par la Mission P. Allorge em 1936. Société D'Édition d'Enseignement Supérieur, Paris.
- DESIKACHARY, T.V. 1959. Cyanophyta. Indian Council of Agricultural Research, New Delhi.
- FIORE, M.F., SANT'ANNA, C.L., AZEVEDO, M.T.P., KOMÁREK, J., KAŠTOVSKÝ, J., SULEK, J. & LORENZI, A.S. 2007. The cyanobacterial genus *Brasilonema*, gen. nov., a molecular and phenotype evaluation. *Journal of Phycology* 43:789-798.
- KOMÁREK, J. 1989. Studies on the Cyanophytes of Cuba 7-9. *Folia Geobotanica Phytotaxonomica* 24:171-206.
- KOMÁREK, J. 2003. Two *Camptylonemopsis* species (Cyanoprokaryotes) from "Mata Atlântica" in coastal Brazil. *Preslia* 75:223-232.
- NEILAN, B.A., JACOBS, D., DOT, T.D., BLACKALL, L.L., HAWKINS, P.R., COX, P.T. & GOODMAN, A.E., 1997. rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. *International Journal of Systematic Bacteriology* 47:693-697.
- SANT'ANNA, C.L., BICUDO, R.M.T. & PEREIRA, H.A.S.L. 1983. Nostocophyceae (Cyanophyceae) do Parque Estadual da Ilha do Cardoso, Estado de São Paulo, Brasil. *Rickia* 10:1-27.
- STACKEBRAND, E. & GOEBEL, B.M. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic Bacteriology* 44:846-849.
- TAMURA, K., DUDLEY, J., NEI, M. & KUMAR, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596-1599.
- WAYNE, L.G., BRENNER, D.J., COLWELL, R.R., GRIMONT, P.A.D., KANDLER, O., KRICHEVSKY, M.I., MOORE, L.H., MOORE, W.E.C., MURRAY, R.G.E., STACKEBRAND, E., STARR, M.P. & TRÜPER, H.G. 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *International Journal of Systematic Bacteriology* 37:463-464.