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Basicladia emedii (Cladophorales, Chlorophyta): a new freshwater epilithic species from Brazil

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Abstract – *Basicladia emedii* sp. nov. is described based on material from six streams in Paraná and Rio Grande do Sul States of Brazil. This is only the second species of *Basicladia* recorded from South America. The new species is distinguished based on small filaments with short and wide basal cells of upright axes. It is only the second (out of six) species that is only known with an epilithic habit and is not associated with either snail shells or turtle carapaces.

Macroalgae / green algae / filamentous / subtropical / lotic systems / Brazil

Résumé – ***Basicladia emedii* sp. nov. (Cladophorales, Chlorophytes) : une nouvelle espèce épilithique d'eau douce du Brésil.** *Basicladia emedii* sp. nov. est décrit d'après du matériel provenant de six cours d'eau de l'Etat de Paraná et Rio Grande do Sul au Brésil. C'est seulement la deuxième espèce observée en Amérique du Sud. La nouvelle espèce se distingue par sa petite taille associée à des cellules basales courtes et larges. C'est aussi la seconde espèce, sur six, qui n'est connue que comme épilithe et donc qui n'est associée ni à des coquilles de mollusques ni à des carapaces de tortues.

Macroalgues / algues vertes / filamenteuses / subtropical / système lotique / Brésil

INTRODUCTION

The genus *Basicladia* is a member of the order Cladophorales Haeckel, and is characterized by the following features: 1) a heterotrichous thallus, with a basal layer of non-rhizoidal cells and erect axes comprised of siphonocladous, poorly branched filaments; 2) erect axes with cylindrical cells at the base that are several times longer than wide and become shorter and barrel-shaped towards the apex; 3) cell walls thick and lamellate; 4) parietal and reticulated chloroplast, with numerous pyrenoids; 5) moniliform zoidangia in chains at the filament apices; and 6) an amphibious, epizoic habit, *i.e.*, it generally lives on the carapaces of turtles and freshwater snail shells (Hoffman & Tilden, 1930; Castillo, 1997; John, 2002, 2003).

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Basicladia was proposed by Hoffman and Tilden (1930), and *B. crassa* is the type-species of the genus. In the same paper, the authors also included the species *B. chelonum* by the recombination of *Chaetomorpha chelonum* Collins. Later, Smith (1950) transferred *Chaetomorpha sinensis* Gardner to *Basicladia*, Ducker (1958) described *B. ramulosa*, and Normandin and Taft (1959) proposed *B. vivipara*. Except for *B. vivipara*, which was described from material collected from snail shells, the other species occurred primarily on the carapaces of turtles, even though they can be also found as epiphytes. The unusual epizoic habit has been widely used to characterize this genus.

In 1963, van den Hoek created a section in *Cladophora* and named it *Basicladia*, because, according to the author, the presence of a basal layer was not enough to define a distinct genus. Two epilithic cladophoracean species (*C. kosterae* C. Hoek and *C. okamurae* (S. Üeda) C. Hoek) were transferred to the new section, however, the author did not transfer the remaining *Basicladia* species (including the type-species) to the new *Cladophora* section, creating several nomenclatural anomalies. Meanwhile, several studies continued to recognize the taxonomic value of the basal layer, and kept *Basicladia* as a valid and distinct genus (Bourrelly, 1972; Dillard, 1989; Castillo, 1997; John, 2003; Garbary *et al.*, 2007; Skinner *et al.*, 2008; Mrozinska *et al.*, 2009), contrary to the conclusion made by van den Hoek (1963).

Garbary (2010) solved this taxonomic problem based on morphological and molecular evidence presented by Yoshii *et al.* (2004) and transferred the two species of van den Hoek to *Basicladia*, creating *B. okamurae* (S. Ueda) Garbary and *B. kosterae* (C. Hoek) Garbary. The author also confirmed *Basicladia* as a valid genus based on its heterotrichous condition, where the basal layer provided the basis for the primary association with the carapaces of turtles, even though these organisms could survive on other substrata. In addition, an ultrastructural study showed that pyrenoid structure in *Basicladia* differed substantially from *Cladophora* (Mrozinska *et al.,* 2009). Garbary (2010) reconfirmed the genus *Basicladia* and brought the number of species to seven.

The only record of *Basicladia* for Brazilian territory is in Semir *et al.* (1988), where the authors described and illustrated *B. chelonum* from the carapaces of turtles (*Hydromedusa tectifera* and *Phrynops geoffroanus*) from three localities in the State of São Paulo. In this paper we describe a new species of *Basicladia* from Brazil.

MATERIAL AND METHODS

Samples were collected during a comprehensive study of biodiversity of stream macroalgae in conservation units in southern Brazil. This study covered 105 streams of 10 conservation units. *B. emedii* was collected in six sampling sites (two streams in Iguaçu National Park, Paraná State, and four in Turvo Forest State Park, Rio Grande do Sul State).

Specimens were preserved in 4% formaldehyde solutions and taken to the laboratory for study. Examination of the taxonomical characters was conducted using a Leica DM1000 trinocular microscope, and a Leica DFC280 image capture digital camera system. Leica IM-50 image analysis software was used for morphometric analyses. Voucher specimens preserved in 4% formaldehyde were deposited into the SJRP Herbarium at the University of São Paulo

State (UNESP), São José do Rio Preto campus (Holmgren *et al.* 1990), and the *B. emedii* description is in accordance with the new rules adopted in Melbourne for the International Code.

RESULTS AND DISCUSSION

***Basicladia emedii* sp. nov.** **Figs 1-16**

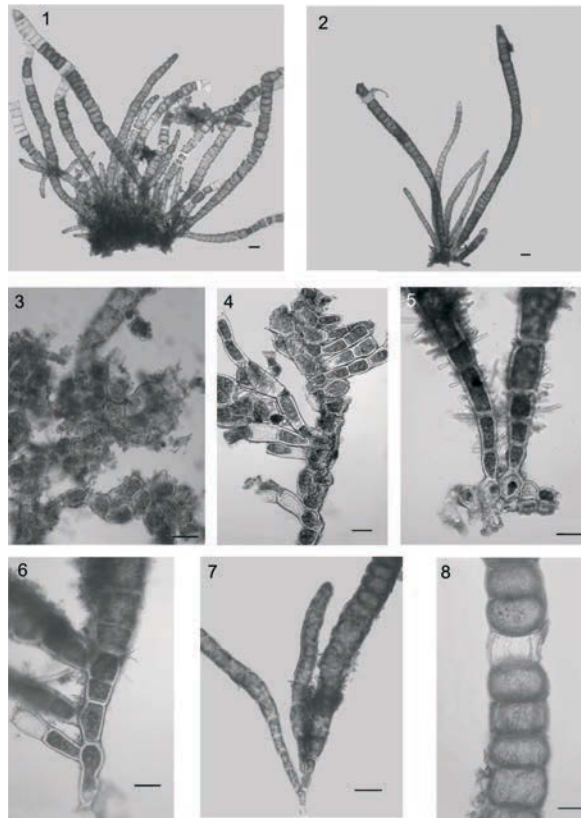
Diagnosis. Small filaments (0.7-3.4 mm), heterotrichous; scarce branches; basal cell with a length of 45.9-121.8 (-198.5) µm and a width of 18.9-46.2 µm; short axis cell with a length of (30.4-) 34.6-131.5 µm and a broad width of 56.8-176.0 µm. This species differs from all others in the genus because it consists of small filaments and has short, wide basal cells of upright axes.

Holotype. BRAZIL, PARANA STATE: Céu Azul municipality, Iguaçu National Park, unnamed stream, 25°09'38"S, 53°49'44"W, 476 m a.s.l., C.C.Z. Branco *et al.*, 02.v.2008, SJRP 29760.

Etymology. The species epithet *emedii* is dedicated to the memory of our phycologist colleague Rafael Guilherme Emed (*08/07/1985, † 12/01/2008) who helped in the collection of the specimens that were examined.

Description. Thallus filamentous, heterotrichous, dark green, usually hosting cyanobacteria and diatoms. Prostrate system formed by branched creeping filaments that frequently coalescing to form a pseudoparenchymatous layer or, in some cases, stolons; creeping filament cells rounded or irregular, thick-walled, often forming rhizoids, (16-)22.1-88.6 µm long ($\bar{x}$ = 49.0 ± 12.5, mean ± s.d.), (14.1) 16.5-63.2(-68.7) µm diam. ($\bar{x}$ = 37.9 ± 9.3), length/diameter 0.6-2.6 ($\bar{x}$ = 1,3 ± 0,3). Erect system formed by short branched filaments, 13-49(-94) cells in length, 730-3386 µm ($\bar{x}$ = 1743 ± 747); basal cell of main axis cylindrical or club-shaped, longer than other axial cells, 45.9-121.8(-198.5) µm long ($\bar{x}$ = 75.0 ± 22.1), 18.9-46.2 µm diam. ($\bar{x}$ = 29.9 ± 5.5), length/diameter 1.5-5.0 ($\bar{x}$ = 2.6 ± 0.7), thick-walled; cells of main axis cylindrical at the base and moniliform at the apex of the filaments, constricted, (30.4-)34.6-131.5 µm long ($\bar{x}$ = 64.0 ± 16.3), 56.8-176.0 µm diam. ($\bar{x}$ = 105.1 ± 25.1), length/diameter 0.3-1.3 ($\bar{x}$ = 0.6 ± 0.2); apical cells cylindrical or acuminate, 35.7-102.5(-124.3) µm long ($\bar{x}$ = 75.0 ± 15.9), (34.8-)40.4-81.8(-98.0) µm diam. ($\bar{x}$ = 61.1 ± 13.9), length/diameter (0.7-)0.8-2.2 ($\bar{x}$ = 1.3 ± 0.3); branches scarce or absent, unilateral or dichotomous, occurring usually on the basal cell up to the third cell of the erect filament and rarely up to the sixth cell, inserted in a subterminal or median position in the branched cell. Chloroplast parietal, reticulate, with numerous pyrenoids. Zoidangia in chains, not differentiated from the other main axis cells, 32.4-106.9 µm long ($\bar{x}$ = 62.4 ± 15.2), 46.6-157.7 µm diam. ($\bar{x}$ = 104.2 ± 25.5), length/diameter 0.4-1.1(-1.5) ($\bar{x}$ = 0.6 ± 0.2), with a single lateral pore located in median position, pore diameter 10-20 µm; zoospore diameter 5.6-11.0 µm ($\bar{x}$ = 10.5 ± 3.2).

Specimens examined. BRAZIL, RIO GRANDE DO SUL STATE: Derrubadas municipality, Turvo Forest State Park, Calisto river, 27°13'49"S, 53°54'92"W, 217 m a.s.l., C.C.Z. Branco *et al.*, 17.viii.2007, SJRP 29739; *ibid*, Bonifácio river, 27°12'24"S, 53°50'01"W, 220 m a.s.l., C.C.Z. Branco *et al.*, 18.viii.2007, SJRP 29740; *ibid*, Tigre river, 27°12'25"S,

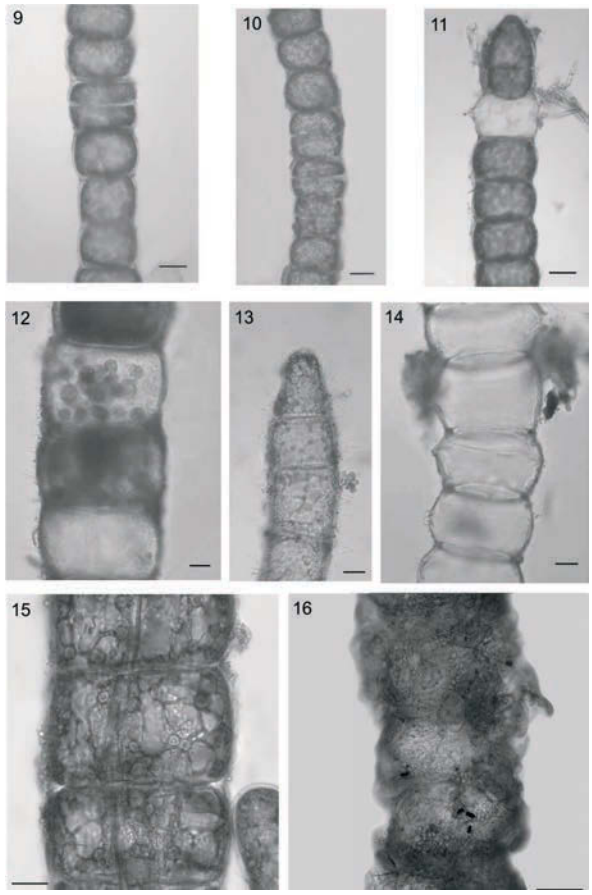


Figs 1-8. *Basicladia emedii.* **1-2.** Entire thallus. **3-4.** Prostrate system. **5.** Prostrate system and basal cells. **6-7.** Branching pattern and basal cell. **8.** Axis cells with a zoidangium. (Scale bar = 100 μm for Figs 1-2, 7; and 50 μm for Figs 3-6, 8).

53°50'02"W, 227 m a.s.l., C.C.Z. Branco *et al.*, 18.viii.2007, SJRP 29741; *ibid*, unnamed stream, 27°11'57"S, 53°49'31"W, 220 m a.s.l., C.C.Z. Branco *et al.*, 18.viii.2007, SJRP 29742; BRAZIL, PARANÁ STATE: Foz do Iguaçu municipality, Iguaçu National Park, at the confluence of the Apepu and Apepuzinho rivers, 25°32'03"S, 54°18'08"W, 194 m a.s.l., C.C.Z. Branco *et al.*, 30.iv.2008, SJRP 29754; *ibid*, Céu Azul municipality, Iguaçu National Park, unnamed stream, 25°09'38"S, 53°49'44"W, 476 m a.s.l., C.C.Z. Branco *et al.*, 02.v.2008, SJRP 29760.

Taxonomic observations

Although specimens were collected from different regions (Rio Grande do Sul and Paraná States) and distinct environmental conditions, the range of taxonomic characters was similar in different populations. In the description of the genus *Basicladia*, Hoffmann and Tilden (1930) emphasized that basal cells of upright axes were long and gradually became shorter and swollen at the apex, forming zoidangia in the apical region. The characters of *B. emedii* were consistent with this description, although the length of the basal cells of upright axes did not differ substantially from the axial cells in this species.



Figs 9-16. *Basicladia emedii*. **9-10**. Axis cells. **11**. Apical cell and zoidangium. **12**. Zoidangium with zoospores. **13**. Apical cell. **14**. Empty zoidangia. **15**. Reticulate chloroplasts with pyrenoids and **16**. Epiphytism by cyanobacteria and diatoms. (Scale bar = 50 µm for Figs 9-11, 13, 16; and 20 µm for Figs 12, 14-15).

Basicladia emedii differs from the other species of the genus in several aspects (Table 1). Specimens examined in this study can be distinguished from the most common and widely distributed species, *B. chelonum* and *B. vivipara*, because of their broader axial cells, and from other species because of their smaller size. Moreover, *B. emedii* has few and simple branches, and the basal cell of upright axes is always short (less than 200 µm). This differs from *B. ramulosa* and *B. kosterae* where branches are more numerous, and are sometimes bi- or trifurcate, and from *B. crassa*, *B. okamurae* and *B. kosterae* where basal cells are usually longer than 500 µm.

Basicladia emedii is similar to those species with larger filaments, such as *B. crassa*, *B. sinensis* and *B. okamurae*. In addition to the broader axial cells, these species have in common an erect system that is unbranched to poorly branched. When branching is more proliferous, it is usually restricted to the basal cells of upright axes. *Basicladia okamurae* was also recorded as epilithic, as *B. emedii*, but the geographic distribution of these species is quite different (Table 1).

Table 1. Comparison of the diagnostic features between *Basicladia* species and *B. emedii*

Features	*Species*							
	B. chelonum [1]	*B. crassa* [1]	*B. vivipara* [2]	*B. sinensis* [3]	*B. ramulosa* [4]	*B. kosterae* [5]	*B. okamurae* [5]	*B. emedii*
Thallus	Erect and prostrate systems	Erect and prostrate systems	Erect and prostrate systems	Erect and prostrate systems	Erect and prostrate systems	Erect and prostrate systems	Rhizome that originates both the prostrate and erect systems	Erect and prostrate systems
Erect filaments length	10-20 mm	10-20 mm	0,30-0,35 mm	80-120 mm	?-100 mm	?-25, $\bar{x}$ = 10 mm	?-20 mm	0,7-3,5 mm
Basal cell	L: ?-1000 µm, W: 12-20 µm, L/W: ?-50	L: 1325-3175 µm, W: 50-120 µm, L/W: ?-30	L: ?, W: 5-10 µm, L/W: ?		L: 700–2190 µm, W: 30–54 µm, L/W: 21-40	L: 550-1650 µm, W: 27-58 µm, L/W: 9,2-26	L: 1290-6150 (-12000) µm, W: 15-89 µm, L/W: 29-49 (-163)	L: 45,9-121,8 (-198,5) µm, W: 18,9-46,2 µm, L/W: 1,5-5,0
Axis cell	L: ?, W: ?-35 µm, L/W: 5-10	L: 65-125 µm, W: 70-125 µm, L/W: 4-8	L: ?, W: 5-28 µm, L/W: ?	L: ?, W: 60-95 µm, L/W: ?	L: 270-493 µm, W: 29-47 µm, L/W: 6,1-?	L: 105-270 µm, W: 53-85 µm, L/W: 1,7-5,3	L: 100-890 µm, W: 37-98 µm, L/W: 2,2-14,7	L: (30,4-)34,6-131,5 µm, W: 56,8-176,0 µm, L/W: 0,3-1,3
Apical cell	L: ?, W: ?, L/W: 2-3	L: 60-275 µm, W: 30-95 µm, L/W: 1,5-3	L: ?, W: ?, L/W: 2-3		L: 50-106 µm, W: 11,5-19 µm, L/W: 3,5-6,9	L: 74-230 µm, W: (18-)25-38 (-42) µm, L/W: (1,5-)2,9-6,0	L: 93-235 µm, W: 30-77 µm, L/W: 2,2-5,5	L: 35,7-102,5 (-124,3) µm, W: (34,8-)40,4-81,8(-98,0) µm, L/W: (0,7-)0,8-2,2
Zoidangia	L: ?, W: ?-50 µm, L/W: 1-4	L: 87-197 µm, W: 64-127 µm, L/W: 1-1,5	L: ?, W: ?-50 µm, L/W: 1-4		L: 55-116 (-145) µm, W: 17-32 µm, L/W: ?	L: 68-135 µm, W: 70-110 µm, L/W: 0,7-1,8	L: 93-235 µm, W: 30-77 µm, L/W: 2,2-5,5	L: 32,4-106,9 µm, W: 46,6-157,7 µm, L/W: 0,4-1,1(-1,5)
Branching frequency and position	Few branches, especially near the base	Few branches, especially near the base	No branches		Many branches, throughout the axis, bi or trifurcated	Usually many branches, among the third to sixth cell, bifurcated	Many branches, limited to the basal cell	Few branches, especially near the base
Habitat	Turtle shells, epilithic	Turtle shells, epiphytic in wood	Snail shells	Turtle shells	Turtle shells	Turtle shells, epilithic	Epilithic	Epilithic
Distribution	USA, Canada, Brazil, Cuba, Hawai	USA, Mexico	USA	USA	Australia	France, Mexico, Australia	France, Japan	Brazil

1. From original generic description, except length of *B. chelonum* taken from Semir *et al.* (1988) for Brazilian material.
2. From original description, Normandin & Taft (1959); 3. From original description, Normandin & Taft (1959).
4. Measurements of the type carried out by Castillo (1997), except for zoidangia where data taken from Skinner *et al.* (2008).
5. Measurements performed by Castillo (1997).

Garbary (2010) argued that both species listed by Guiry & Guiry (2007) with a provisional status (*B. sinensis* and *B. vivipara*) required further study because neither of the species had been re-collected since their first descriptions, and they were possibly related to *B. okamurae*. In this context, further molecular studies to evaluate the relationship of the *Basicladia* species each other and with other Cladophorales are strongly recommend.

Associated macroalgae species

Cyanobacteria: *Lyngbya majuscula* (Dillwyn) Harvey, *Microcoleus subtorulosus* (Brébisson) Gomont *ex* Gomont, *Nostochopsis lobatus* Wood *ex* Bornet *et* Flahault, *Phormidium retzii* (C.Agardh) Kützing *ex* Gomont, *Phormidium* sp., *Tolypothrix robusta* Gardner; **Chlorophyta**: *Spirogyra* sp.; **Rhodophyta**: *Kumanoa ambigua* (Montagne) Entwisle *et al.*, *B. arcuatum* Kylin, *Hildenbrandia angolensis* Welwitsch *ex* West *et* G.S. West, 'Chantransia' stage; **Heterokontophyta**: *Hydrosera whampöensis* (Schwartz) Deby, *Pleurosira laevis* (Ehrenberg) Compère, *Vaucheria* sp.

Habitat characteristics of *B. emedii*

Specimens were collected under the following environmental conditions (n = 6): temperature 15.3-18.1°C ($\bar{x} = 16.6 \pm 1.2$), specific conductivity 20-46 mS·cm-1 ($\bar{x} = 33 \pm 10$), pH 6.2-7.3 ($\bar{x} = 6.9 \pm 0.4$), dissolved oxygen 4.6-6.0 $mg \cdot l^{-1}$ (= 5.4 ± 0.6), turbidity 2-38 NTU ($\bar{x} = 15 \pm 16$), current velocity 14-102 $cm \cdot s^{-1}$ ($\bar{x} = 56 \pm 34$), average depth 13-24 cm ($\bar{x} = 17 \pm 4$), orthophosphate 0.06-0.18 $mg \cdot l^{-1}$ ($\bar{x} = 0.12 \pm 0.05$), total nitrogen 0.1-1.2 $mg \cdot l^{-1}$ (= 0.7 ± 0.4) and, intermediate riparian shading.

In this study, *B. emedii* was always found on rocky surfaces. The macroalga was found only in shallow streams, particularly at the margins, where variable water levels provide an amphibious condition for macroalgae.

Although sampling was conducted from 105 streams from the four major biomes of southern Brazil, *B. emedii* was only recorded in Seasonal Forest fragments in the Atlantic Forest domain. This fact apparently indicates a preference for this type of environment or region.

ARTIFICIAL KEY TO IDENTIFICATION OF *BASICLADIA* SPECIES

1. Erect system with abundant bifurcate or trifurcate branches along the whole axis 2
   Erect system with scarce or absent branches; when abundant, restricted to the basal cells 3
2. Axial cells longer than 270 µm, bi or trifurcated branches throughout the axis . . . . . *B. ramulosa*
   Axial cells shorter than 270 µm, bifurcated branches from the third to sixth axial cell . . . . . *B. kosterae*
3. Axial cells narrower than 35 µm;, basal cells narrower than 20 µm 4
   Axial cells broader than 37 µm; basal cell usually broader than 20 µm in width 5

4. Basal cells of upright axes narrower than 10 μm; erect filaments shorter than 0.35 mm. . . . . *B. vivipara*
   Basal cell of upright axes broader than 12 μm; erect filaments longer than 10 mm . . . . . *B. chelonum*
5. Erect filaments longer than 10 mm; basal cells longer than 1000 μm 6
   Erect filaments shorter than 3.5 mm; basal cell shorter than 200 μm. . . . . ***B. emedii***
6. Rhizomes present . . . . . *B. okamurae*
   Rhizomes absent 7
7. Filaments 10-20 mm long. . . . . *B. crassa*
   Filaments 80-120 mm long. . . . . *B. sinensis*

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