

# Morphology of the larval shell of three oyster species of the genus *Crassostrea* Sacco, 1897 (Bivalvia: Ostreidae)

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(With 8 figures)

## Abstract

In this study we describe the morphology of the larval shell of three oyster species of *Crassostrea* genus. Two species, *C. rhizophorae* and *C. brasiliiana*, are native to the Brazilian coast, and *C. gigas* is an introduced species. Samples of laboratory reared larvae, obtained through artificial fertilisation, were collected at intervals during the cultivation process for analysis using Scanning Electron Microscopy (SEM). Prodissoconch morphology was observed in relation to the presence, position, form and number of teeth in the three larval stages: D-shaped larva, umbo larva and pediveliger. Characteristic of D-shaped larvae of *C. rhizophorae* was the total absence of teeth in the provinculum area while *C. brasiliiana* and *C. gigas* had two anterior and two posterior teeth in each valve. In the umbo larval phase, the three species had the same number of teeth in each valve: two posterior and two anterior teeth in the right valve and three posterior and three anterior in the left valve. In the pediveliger stage the three species could be differentiated by the number of anterior teeth of the right valve: *C. rhizophorae* had two teeth, *C. brasiliiana* one tooth and *C. gigas* three teeth.

*Keywords:* *Crassostrea*, oyster larvae, prodissoconch, shell morphology.

## Morfologia de conchas larvais de três espécies de ostras do gênero *Crassostrea* Sacco, 1897 (Bivalvia: Ostreidae)

## Resumo

Neste estudo, foi descrita a morfologia das conchas larvais de três espécies de ostras do gênero *Crassostrea*. Duas espécies, *C. rhizophorae* e *C. brasiliiana*, são nativas da costa brasileira e *C. gigas* é uma espécie introduzida. Amostras de larvas produzidas em laboratório através de fertilização artificial foram coletadas em intervalos durante o processo de cultivo para análises dos número de dentes nos três estágios larvais: larva D, umbo e pediveliger. As larvas D de *C. rhizophorae* caracterizaram-se pela total ausência de dentes na área do provinculum, enquanto *C. brasiliiana* e *C. gigas* apresentaram dois dentes anteriores e dois posteriores em cada valva. Na fase umbo, as três espécies apresentaram o mesmo número de dentes em cada valva: dois dentes anteriores e dois posteriores na valva direita; e três dentes posteriores e três anteriores na valva esquerda. Na fase pediveliger, as três espécies se diferenciaram pelo número de dentes anteriores na valva direita: *C. rhizophorae* apresentou dois dentes; *C. brasiliiana*, um dente; e *C. gigas*, três dentes.

*Palavras-chave:* *Crassostrea*, larvas de ostra, prodissoconcha, morfologia.

## 1. Introduction

Oysters are bivalve mollusks, belonging to the family Ostreidae, with wide geographical distribution. The genus *Crassostrea* Sacco, 1897 comprises species of great economic importance. They occur from estuary zones of low salinity, as in the case of the Brazilian species *C. rhizophorae* (Guilding, 1828) and *C. brasiliiana* (Lamarck, 1819), to areas of normal marine salinities, including the Japanese oyster *C. gigas* (Thunberg, 1793) which is widespread and cultivated in several countries (Malouf and Brees, 1977). The presence of two species of oysters of the *Crassostrea* genus on the Paraná Coast (Brazil), has been confirmed by various authors (Absher, 1989; Ignácio et al., 2000; Lazoski, 2004) through comparative studies based on aloenzymes analysis, though *C. rhizophorae* and *C. brasiliiana*, are still considered by some authors (e.g. Rios, 2009) as synonymous. *Crassostrea rhizophorae*, inhabits the intertidal area, fastened to the aerial roots of mangrove reaching at the most 8-9 cm in height (Nascimento, 1983). This species occurs from the Caribbean waters to Uruguay (Rios, 2009). *Crassostrea brasiliiana* may reach more than 25 cm in height (Absher, 1989). Larvae of both species are present at the same time in plankton samples from the area, since these species reproduce throughout the year (Absher, 1989; Absher et al., 2000).

The dimension of the larval shell of oysters, its general form, the prominence and form of the umbo during the planktonic period, and the relationships between the length and height of the provinculum, are important diagnostic characteristics (Dinamani, 1976; Waller, 1981; Hu et al., 1993; Carriker, 1996; Eble and Scro, 1996). Thus, detailed studies of the morphological characteristics of the larval shell allow the description and differentiation of congeneric species.

Detailed studies of the larval shell using Scanning Electron Microscopy (SEM) supply information about the modifications that occur in the provinculum of the prodissoconch in the course of larval and post-larval development. It is important to differentiate con-generic larvae of Ostreidae and of larvae of other bivalve families, when isolated in planktonic samples (Webb, 1987; Fuller et al., 1989; Hu et al., 1993; Kimura and Sekiguchi, 1994; Monteiro-Ribas et al., 2006). The first larval stage after the trocophore of bivalves is characterised by the beginning of calcification and the formation of a shell-prodissoconch I (PI) followed by the prodissoconch II (P-II), with different structures characteristic of each species (Christo and Absher, 2008). Micrography produced using SEM of the larval shells of oysters can provide important information about the modifications of the prodissoconch. The objective of this study was to compare the morphological characteristics of the larval shells between trocophore and pediveliger of *C. rhizophorae*, *C. brasiliiana* and *C. gigas*, as well as to determine diagnostic characters for the identification of their larvae.

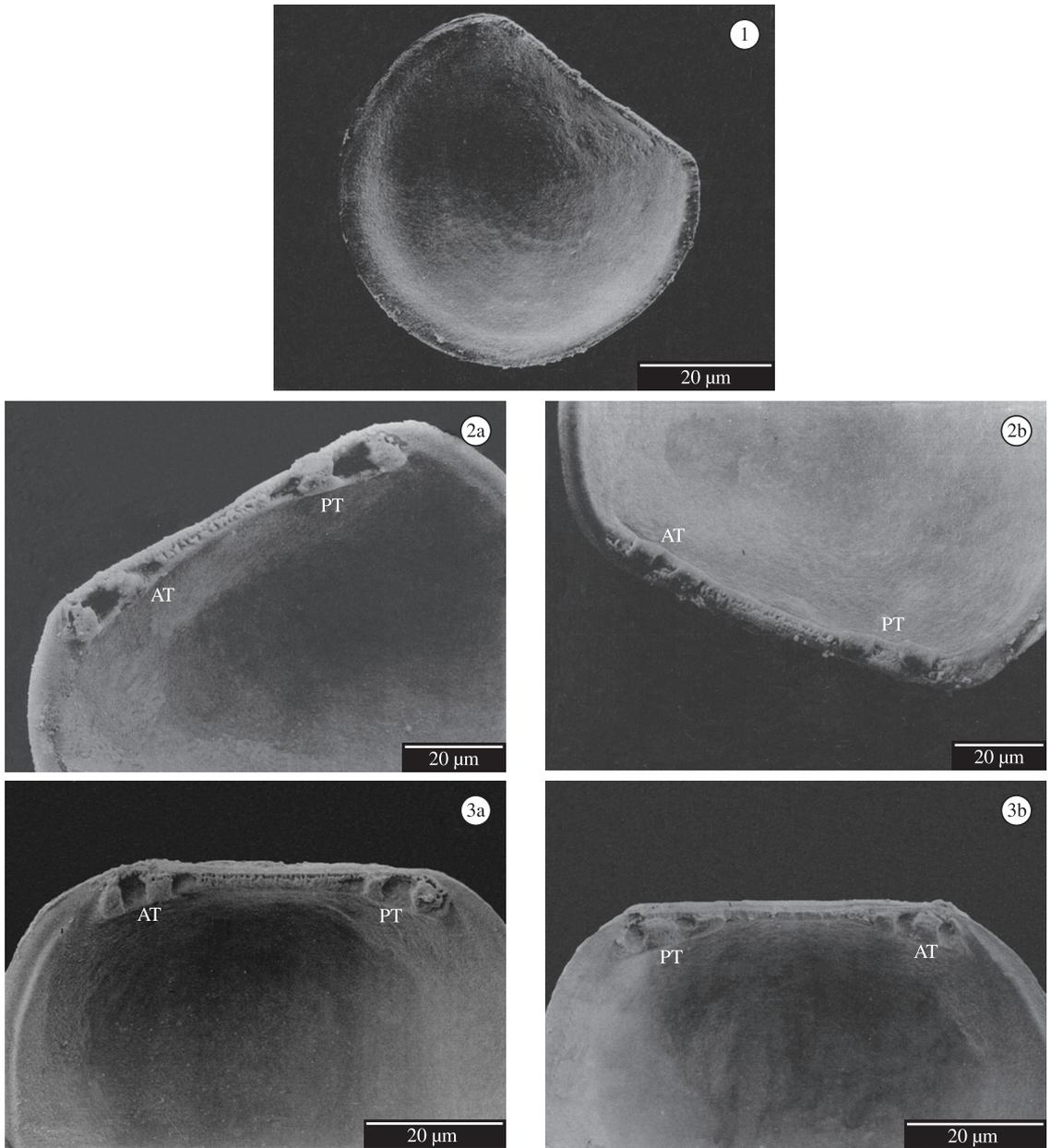
## 2. Material and Methods

The comparative description of the provinculum of the larval shell was based on disjointed valves of the three oyster species reared in the laboratory from artificial fertilisation. The parent specimens of *C. rhizophorae* and of *C. brasiliiana* were obtained from the coast of the State of Paraná and of *C. gigas* from Santa Catarina State (South Brazil). Specimens with full and/or partially filled gonads were used for the rearing experiments. Reproductive cells were stripped from clean individuals of two females for each male. Rearing techniques were adapted from Absher et al. (2000). During the whole cultivation process, samples of larvae were collected and preserved in ethanol 70% and later transferred to a solution of sodium hydroxide (5-10%) and left for approximately 48 hours, before the separation of the valves. After this period, the shells were washed with 90% ethanol, dried at 65 °C for about 10 minutes and mounted on adhesive ribbon in metallic supports, for metallisation through vaporisation (i.e., sputter coating) with gold for the exam of the larval shell in the scanning electron microscope. Observations and electron micrographs were performed and obtained on Philips XL – 30 equipment of LAC/CNMT – COPEL. Height measurements (dorso-ventral axis) of the larvae were obtained during the first phase of each larval stage.

## 3. Results

In the phase of D-shaped larva, the prodissoconch I area of the hinge was a completely straight line in all the species. The height of the larvae in that phase varied from 51 µm (*C. rhizophorae*) to 73 µm (*C. gigas*). A total absence of teeth in this area of the right and left valves characterises the *C. rhizophorae* larvae (Figure 1). *Crassostrea brasiliiana* larvae presented in the hinge line two rectangular teeth of the same size in the anterior and posterior extremities of the right and left valves (Figure 2a-2b, respectively), as did *C. gigas* (Figure 3).

Umbo larvae were characterised by a sloping form in the direction of the posterior end of the shell, due to the development of the left valve umbo in the three species. The right valve of *C. rhizophorae* larvae of 115 µm height had two anterior and of two posterior teeth of the same size (Figure 4a). Three anterior and three posterior teeth were developed in the left valve, and the central tooth was more conspicuous than the others (Figure 4b). The two cavities observed in the provinculum of the right valve correspond to the fitting of the teeth of the left valve. *Crassostrea brasiliiana* larval shells of 116 µm in height developed, as described by Absher et al. (2000), two anterior and two posterior teeth of the same size in the right valve and three cavities for the fitting of the left valve teeth. And the left valve showed three anterior and three posterior teeth, with the anterior and posterior distal teeth smaller than the central tooth. Larvae of *C. gigas* of 152 µm in height had



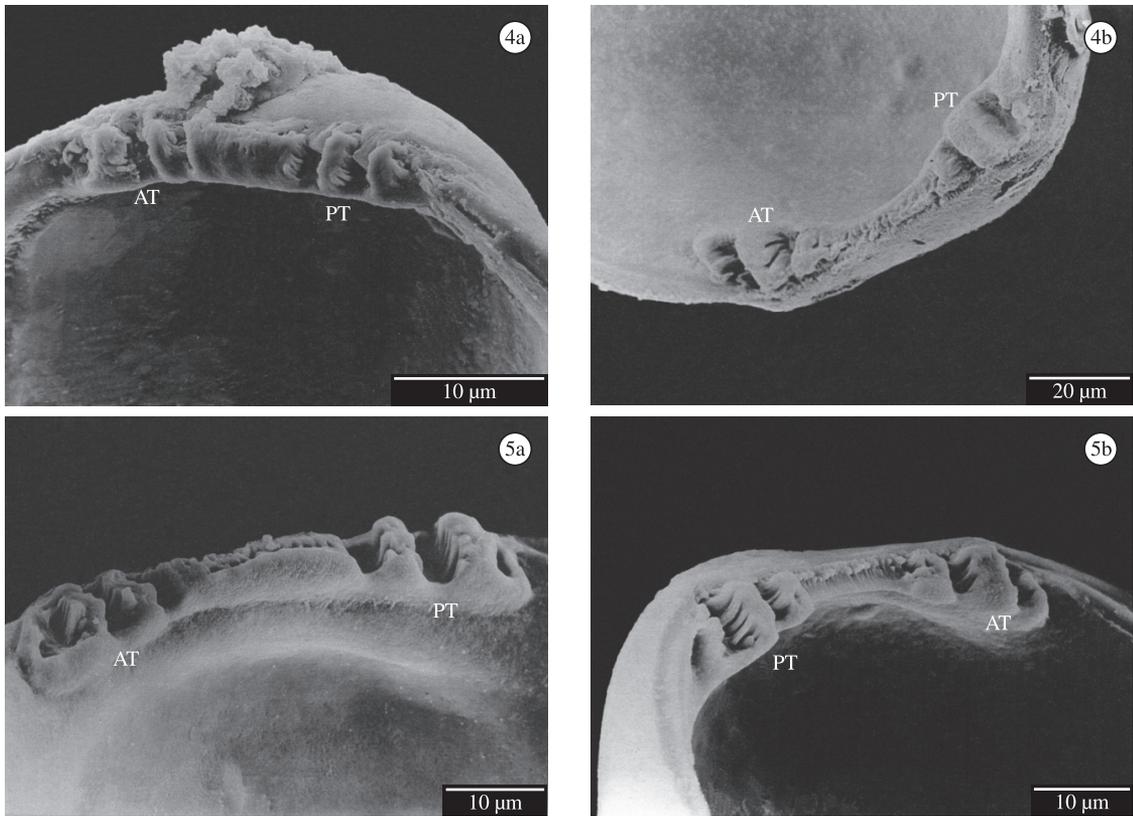
**Figure 1-3.** SEM micrography of larval shells of oysters in D-shaped Stage. 1) *C. rhizophorae*, indicating the absence of teeth in the provinculum; 2) *C. brasiliana*, characterised by anterior teeth (AT) and posterior teeth (PT); 3) *C. gigas*, with anterior teeth (AT) and posterior teeth (PT). a) right valve; b) left valve.

two anterior and two posterior teeth and three cavities in the right valve, corresponding to the fitting of the teeth of the left valve (Figure 5a). This species was characterised by the presence of three anterior and three posterior teeth in the left valve, with the central tooth more conspicuous than the distal teeth (Figure 5b).

During the pediveliger phase, when the larval shells reach a height of more than 300 µm, on average, except in *C. gigas*, a strong curvature of the umbo develops on the hinge towards the posterior end, hiding the posterior teeth of the left valve. Larvae of *C. rhizophorae* with

height of 337 µm presented two anterior teeth in the right valve (Figure 6a) and one anterior tooth in the left valve (Figure 6b). *Crassostrea brasiliana*, with height of 390 µm, was characterised by the presence of one anterior tooth in the right valve (Figure 7a) and two anterior teeth in the left valve (Figure 7b); while *C. gigas* larvae, with a height of 379 µm, presented three and two anterior teeth in the right and left valves, respectively (Figure 8a-8b).

Table 1 summarises the measurements of the larval stages and the differences in the provinculum area in teeth number of the three species studied, in all phases of larval growth.



**Figure 4-5.** SEM micrography of larval shells of oysters in umbo Stage. 4) *C. rhizophorae*; 5) *C. gigas*. a) right valve; b) left valve; AT – anterior teeth; PT – posterior teeth.

#### 4. Discussion

The morphology of the proviculum area of the three species of oysters, reported in this paper, is an important auxiliary tool for the identification of larvae in plankton samples. It should also be of use in confirming the success of laboratory produced oyster larvae from parents of uncertain identification.

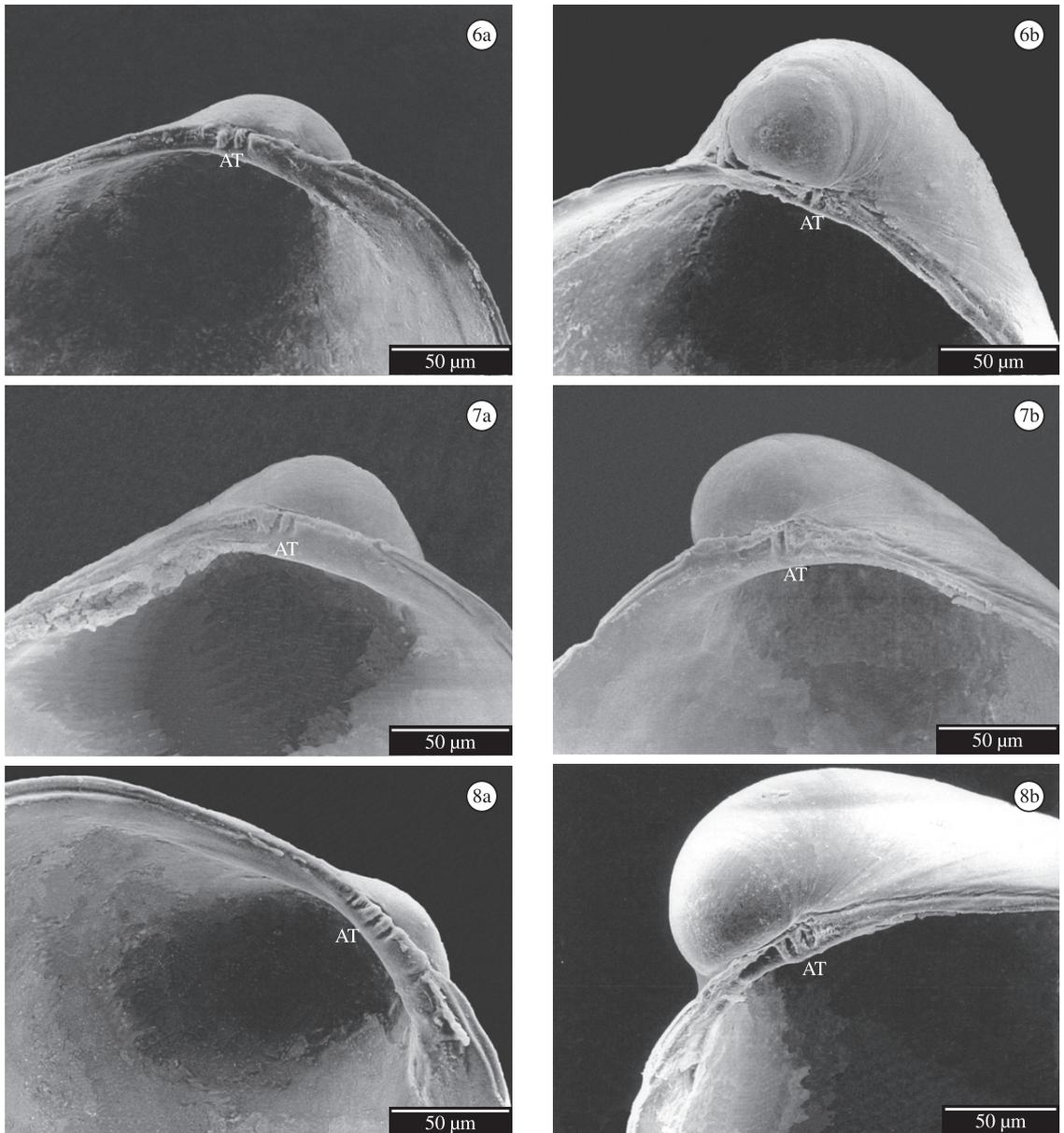
The straight-hinged D-shaped shell of *C. rhizophorae* larvae can be identified by the total absence of teeth in the proviculum area, while larvae of *C. brasiliana* and *C. gigas* are indistinguishable, because of the presence of the same number of teeth in each extremity of the proviculum of both valves.

The umbo area of larvae larger than 110 μm, showed a strong growth of the umbo above the level of the hinge, curved towards the posterior direction – Opistogiro. This characteristic, reported by Dinamani (1976) for *C. gigas*, *C. angulata* and *C. virginica* and by Ver (1986) for *C. iredalei*, is only observed in *Crassostrea* larvae. In this phase of larval development, in *C. brasiliana* and *C. gigas*, a third distal tooth was observed to form in both extremities of the left valve. However there is no differentiation among the three studied species, as to the number, forms and size of the teeth in the proviculum area, in the umbo phase. The left valve of all the species presents three teeth, with the middle teeth more conspicuous than the distals. The variation observed in the length of the proviculum of

the left valve, larger than the right valve, for the studied species is a consequence of the appearance of the distal teeth in the left valve. This increase in the length of the proviculum from this larval phase was also observed by Hu et al. (1993).

During the pediveliger stage, when the larvae reach a height above 300 μm, the posterior teeth of the left valve disappear in consequence of a new shell deposit in the umbo area and the “turn” of this toward the posterior direction. This reduction of the teeth in the posterior area, common in species of *Crassostrea* (Dinamani, 1976; Hu et al., 1993) was observed in this study, in *C. rhizophorae* larvae of 337 μm, in *C. brasiliana* of 390 μm and in *C. gigas* of 379 μm height. The anterior teeth of the left valve and of the right valve are present and vary in number among the studied species. The posterior teeth of the right valve disappear due to the absence of the teeth of the left valve.

Thus, the results of the comparative studies of the morphology of the prodissoconch of *C. rhizophorae*, *C. brasiliana* and *C. gigas*, indicated in D-shaped larvae the absence of teeth in the proviculum only in *C. rhizophorae*, and that in the umbo larval phase the same number of teeth were observed in the three studied species. Larvae in the pediveliger stage present different numbers of teeth in the right valve, allowing the identification of the three species in this stage.



**Figure 6-8.** SEM micrography of larval shells of oysters in pediveliger Stage. 6) *C. rhizophorae*; 7) *C. brasiliana*; 8) *C. gigas*. a) right valve; b) left valve; AT – anterior teeth; PT – posterior teeth.

**Table 1.** Number of teeth and mean height and length of different stages of larval growth of the three species of oysters.

Larval stage	Number of teeth in the provinculum				Species	Mean height (µm) ± SD n = 30
	Right valve		Left valve			
	Anterior	Posterior	Posterior	Anterior		
D – larvae	0	0	0	0	<i>C. rhizophorae</i>	51 ± 4
	2	2	2	2	<i>C. brasiliana</i>	56 ± 0.5
	2	2	2	2	<i>C. gigas</i>	73 ± 3.6
Umbo	2	2	3	3	<i>C. rhizophorae</i>	115 ± 3.7
	2	2	3	3	<i>C. brasiliana</i>	116 ± 4.4
	2	2	3	3	<i>C. gigas</i>	152 ± 10
Pediveliger	2	0	0	1	<i>C. rhizophorae</i>	337 ± 16
	1	0	0	2	<i>C. brasiliana</i>	390 ± 7.2
	3	0	0	2	<i>C. gigas</i>	379 ± 12

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