

Phacus multifacies sp. nov., a new Euglenophyceae from the State of Rio Grande do Sul, Southern Brazil

Sandra Maria Alves-da-Silva^{1,3} and Carlos Eduardo de Mattos Bicudo²

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ABSTRACT - (*Phacus multifacies* sp. nov., a new Euglenophyceae from the State of Rio Grande do Sul, Southern Brazil). As a result of the taxonomic survey of the pigmented Euglenophyceae of Lago da Ponte, an artificial pond located at the Porto Alegre Botanical Garden, State of Rio Grande do Sul, Southern Brazil and samples gathered later on from this same water body, a new species of *Phacus* (Euglenophyceae, Phacaceae), *P. multifacies* Alves-da-Silva & C. Bicudo, sp. nov., is here described and proposed as new to science. Cell displacement turning ventrally one full turn round itself and also rotating around its longitudinal axis and the presence of a dorsal wing-like expansion next to the caudal process are the main diagnostic features of the new species. Physical and chemical information on the pond water is presented.

Keywords: abiotic variables, Phacaceae, *Phacus multifacies* sp. nov., taxonomy, Brazil

RESUMO - (*Phacus multifacies* sp. nov., uma nova Euglenophyceae do Estado do Rio Grande do Sul, Sul do Brasil). Como resultado do levantamento taxonômico das Euglenophyceae pigmentadas do Lago da Ponte, um sistema artificial situado no Jardim Botânico de Porto Alegre, Rio Grande do Sul, Sul do Brasil e de amostras coletadas posteriormente do mesmo ambiente foi identificada uma espécie de *Phacus* Dujardin 1841 emend. Linton & Karnkowska 2010 (Euglenophyceae, Phacaceae), *P. multifacies* Alves-da-Silva & C. Bicudo, sp. nov., a qual é descrita e proposta como nova para a Ciência. O tipo de deslocamento do indivíduo girando ventralmente um turno completo sobre si mesmo e também ao longo de seu eixo longitudinal e uma expansão aliforme dorsal próximo do processo caudal são as principais características diagnósticas da nova espécie. São também fornecidos valores das variáveis abióticas do ambiente em que a espécie foi coletada.

Palavras-chave: Phacaceae, *Phacus multifacies* sp. nov., taxonomia, variáveis abióticas, Brasil

Introduction

Phacus (Euglenales, Phacaceae) includes something in between 150 (Bourrelly 1970) and 180 infrageneric taxa (Huber-Pestalozzi 1955), with worldwide distribution mainly in shallow, warm, β to σ -mesosaprobic freshwater environments. Guiry & Guiry (2017) mentioned the existence of 566 species and infraspecific names, from which 171 are currently taxonomically accepted.

Most *Phacus* species are euplanktonic, occurring together with species of other Euglenid genera (mainly *Euglena*, *Lepocinclis* and *Trachelomonas*), and certain species are capable of surviving high nutrient concentrations. Unlike *Euglena* and *Trachelomonas*, *Phacus* rarely becomes a major component of phytoplankton communities (Weik 1967). Its

representative individuals are unicellular and free-swimming due to the presence of two flagella, from which just one emerges from the reservoir. The cell is naked, *i.e.* do not have a cell wall, but a rigid pellicle with longitudinal or helicoidal striae; cell is dorsiventrally flattened, has the shape variable between fusiform and ovate, but some have wing like projections and the posterior pole with a caudal process of variable length depending on the species. Vertical view may be elliptic, sausage-shaped, somewhat triangular or triradiate.

Paper is a result of the taxonomic survey of the pigmented Euglenophyceae of the Lago da Ponte located at the Porto Alegre Botanical Garden, State of Rio Grande do Sul, Southern Brazil. It aimed at describing and proposing a new species of *Phacus*, *P. multifacies* Alves-da-Silva & C. Bicudo.

1. Fundação Zoobotânica do Rio Grande do Sul, Museu de Ciências Naturais, Seção de Botânica de Criptógamas, Rua Dr. Salvador França 1427, 90690-000 Porto Alegre, RS, Brasil
2. Instituto de Botânica, Avenida Miguel Estéfano, 3.687, 04045-972 São Paulo, SP, Brasil
3. Corresponding author: salvesilva5@yahoo.com.br

Material and methods

The Porto Alegre Botanical Garden is located at 30°03'05"S and 51°10'34"W, covers an area of ca. 39 ha, and includes two artificial ponds. The one presently studied is locally known as Lago da Ponte, but it was a wetland before conversion into a pond in 2003 after removal of the grass-like vegetation and addition of water. Building of the pond aimed only at landscaping composition. Presently, the pond is very much anthropized and mainly maintained by rain water. Its surface is at present covered with *Salvinia* sp. Fish (grass carps), black swans, turtles and terrapins are the pond main inhabitants.

Monthly sampling was performed between July 2007 and June 2008 in two opposite stations located at the pond margin, and later on at a daily basis between 12 and 15 and 18 and 19 January 2010 only at station 1 using a 25 µm mesh plankton net. After collection, samples were immediately fixed and preserved with 4% formaldehyde water solution. Water and air temperature, pH (pHmeter DMPH-P), sampling stations depth, water transparency (Secchi disk), and electric conductivity (conductivimeter Digimed, model CD-28) were measured 'in situ'; dissolved oxygen, percentage of dissolved oxygen and amount of organic matter in the water were measured in the laboratory using APHA (2005) methods. Analysis of biological material was performed between slide and cover slip using both live and preserved material under a Leica-DMLS optical microscope furnished with micrometer ocular. Illustrations were prepared with the help of a camera-lucida coupled to the microscope optical system, and the photomicrographs were taken with a Sony digital camera also coupled to the microscope (Alves-da-Silva *et al.* 2011).

All sample units studied are deposited at the algae collection of Prof. Dr. Alarich R.H. Schultz Herbarium (HAS) of the Natural Sciences Museum of Rio Grande do Sul Zoobotanical Foundation, under the access numbers 107574, 107575, 107576, 107815, 108354, 108355, 108358, 108359, 108362, 108363, 108370 and 108371.

Results and Discussion

Class Euglenophyceae
Order Euglenales
Family Phacaceae

Phacus Dujardin 1841 emend. Linton & Karnkowska 2010

From the 40 sample units studied, individual specimens of *P. multifacies* Alves-da-Silva & C. Bicudo, sp. nov. were found in just 12.

Phacus multifacies Alves-da-Silva & C. Bicudo, sp. nov.

Figures 1-29

Cellula ovata, fortiter asymmetrica, dorsiventraliter complanata, 20-22,5 × 10-14 µm; longitudine/latitudine ratione = 1,4-1,6; aspectu apicali anguste elliptica ad fere circularem; aspectu laterali subrectangulari ad semilunatum (ca. 18 × ca. 7 µm); aspectu frontali obtriangulari, polo anteriori quam posteriori latiori, interdum 1 expansione aliformi dorsali solum ad marginem dexteram poli posterioris; polo posteriori abrupte in processu caudato hyalino, attenuato, acuminato, recto ad leviter obliquo, ad 4 µm longum; pellicula striis longitudinalibus praeditis, granulis paramyllum 3, 2 concentricis, 6-7 µm diam., 1 excentrico, 3-4 µm diam.; chloroplastidiis discoidalibus, numerosis, ca. 2 µm diam., flagello ca. 1-plo cellulae longitudinis.

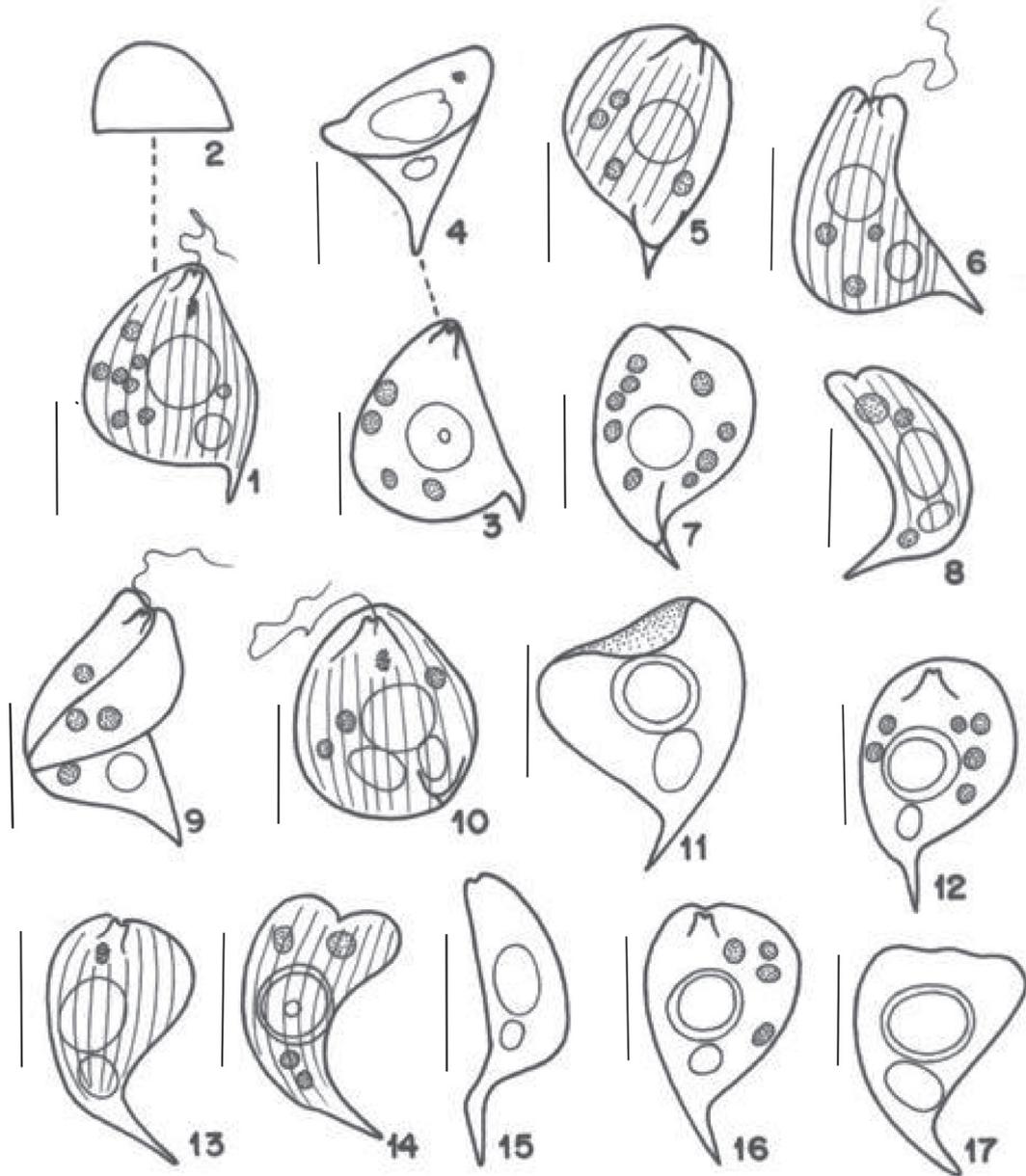
Holotypus: Brasilia, Provincia Rio Grande do Sul, Porto Alegre, Fundação Zoobotânica do Rio Grande do Sul, Lago da Ponte, leg. *F. Friedrich* s.n., V-2008 (HAS107815); figurae 1-4, 8, 11, 17-18, 24, 29.

Cell ovate, strongly asymmetrical, dorsiventrally flattened, 20-22.5 µm long, 10-14 µm broad, 1.4-1.6 times as long as broad; vertical view narrowly elliptic to almost semicircular; lateral view sub-rectangular to lunate (c. 18 µm long, c. 7 µm broad); frontal view obtriangular, anterior pole broader than the posterior, frequently 1 dorsal wing-like expansion only at the right margin of the posterior pole; posterior pole suddenly attenuate into a colorless, acuminate, straight to slightly oblique, up to 4 µm long caudal process; pellicle striate, striae longitudinal, paramylum granules 3, 2 concentric, 6-7 µm diam., 1 eccentric, 3-4 µm diam.; chloroplasts disc-shaped, numerous, c. 2 µm diam., extorted flagellum about as long as the cell length.

Holotype: Brazil, Rio Grande do Sul State, Porto Alegre, Fundação Zoobotânica do Rio Grande do Sul, Lago da Ponte, col. *F. Friedrich* s.n., May/2008 (HAS107815); figures 1-4, 8, 11, 17-18, 24, 29.

Species concept in Euglenophyceae is yet essentially morphological. Compared to the total estimated number of species in the group, especially those of *Phacus*, there still are few publications covering molecular biology.

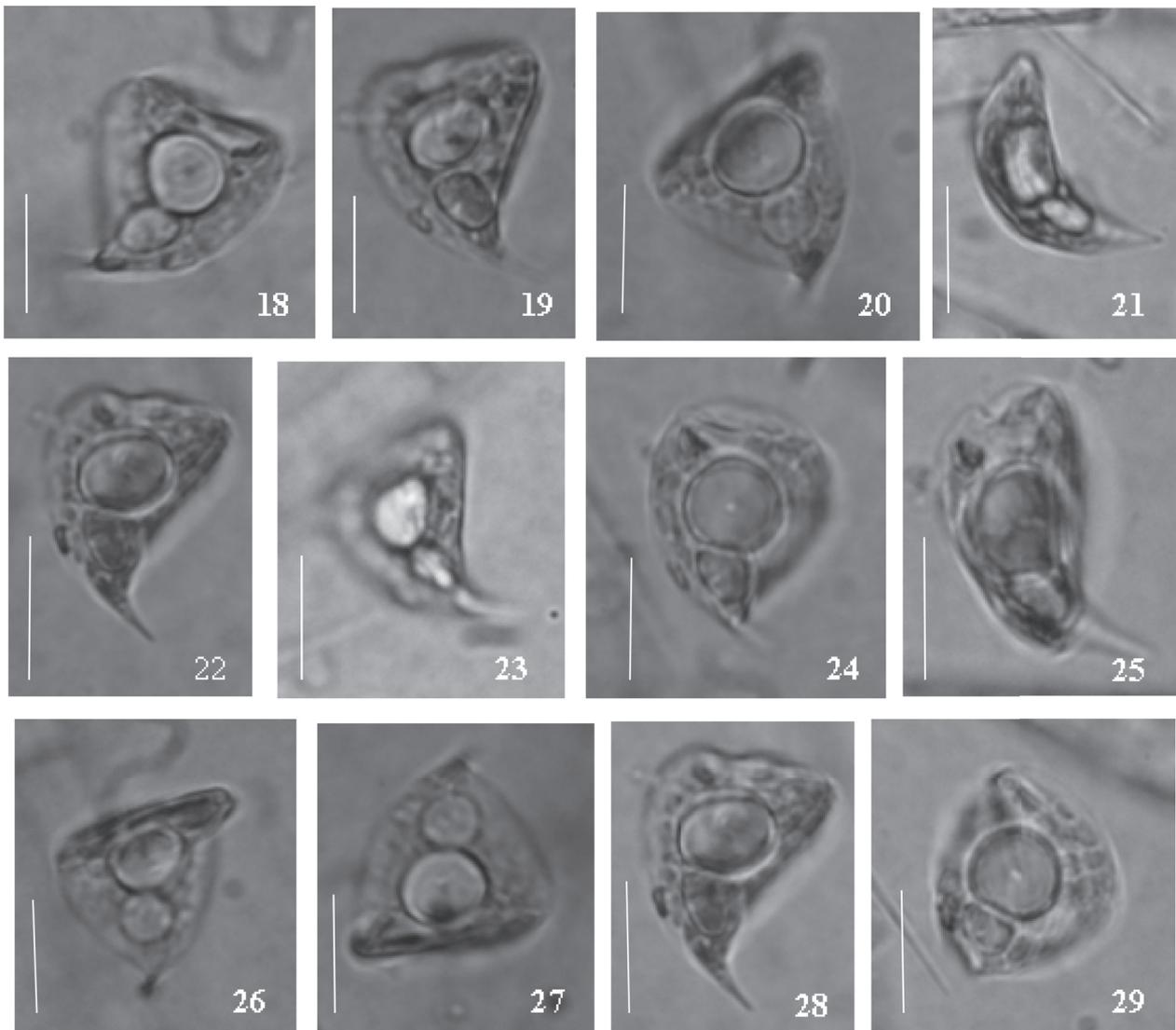
Several papers were published during the last two decades using molecular biology to



Figures 1-17. *Phacus multifacies* Alves-da-Silva & C. Bicudo, sp. nov. 1-17. Camera-lucida line drawings, 2, 4. apical view. 6, 8, 9, 15. Lateral (taxonomic) view. Scale bar = 10 μ m.

elucidate taxonomic and phylogenetic questions on Euglenophyceae (Alves-da-Silva & Bicudo 2009). Molecular biology proved effective in separating genera or in transferring species from one genus to another. According to Pochmann (1942), it would be necessary to treat the *Phacus* section *Pleuraspis* Pochmann as a separate genus, what was done by Marin *et al.* (2003) after revising the plastid-containing euglenophytes on the bases of their SSU rDNA sequences. The entire section was then transferred to the neglected genus *Monomorphina*

Mereschkowsky 1877. Simultaneously and also based on the SSU rDNA sequences, Marin *et al.* (2003) transferred *Phacus agilis* Skuja to *Cryptoglena* and the combination *C. skujae* Marin & Melkonian was used since *Cryptoglena agilis* was already pre-occupied by *Cryptoglena agilis* Ehrenberg. Finally, four *Euglena* species, *E. acus* (O.F. Müller) Ehrenberg, *E. spirogyra* Ehrenberg, *E. oxyuris* Schmarida and *E. spiroides* Lemmermann were transferred to *Lepocinclis* [*L. acus* (O.F. Müller) Marin & Melkonian, *L. spirogyroides* Marin & Melkonian, *L. oxyuris* (Schmarida) Marin



Figures 18-29. *Phacus multifacies* Alves-da-Silva & C. Bicudo, sp. nov. Photomicrographs. 21, 23, 25. Lateral (taxonomic) view. Scale bar = 10 μ m.

& Melkonian and *L. spiroides* (Dujardin) Marin & Melkonian, respectively]; and *Phacus tripteris* Dujardin was transferred to *Lepocinclis* under the combination *L. tripteris* (Dujardin) Marin & Melkonian (Marin *et al.* 2003).

Kosmala *et al.* (2005, 2007a, 2007b, 2009), Triemer *et al.* (2006), Ciugulea *et al.* (2008) and Kim *et al.* (2013) are published attempts to validate the taxonomical changes proposed by Marin *et al.* (2003). Various papers were published in the last two decades describing new species and/or infraspecific taxa of euglenophytes without, however, adding information to their molecular biology (Alves-da-Silva & Bicudo 2003, 2006, Domitrovic & Conforti 2005, Zongo *et al.* 2006, Conforti 2009, 2010, Da & Couté 2009, Tolia

et al. 2012, Duangjan & Wołowski 2013), however, the ones by Kosmala *et al.* (2009) and Kim & Shin (2014) being the only exceptions.

Although molecular studies with this new species material have not been carried out, their morphological characteristics and a distinct displacement way constitute the base for this proposal. Also, worth mentioning is that approximately 80 individuals were found in the 12 sample units studied.

The present new species shows a broad spectrum of cell form variation depending on which position the cell is observed. In lateral view, *P. multifacies* sp. nov. could be somewhat confused with *P. raciborskii* Drezepolski, since it may also be lunate or show a torsion at the anterior pole (figura 9). In lateral

(taxonomic) view it is, however, very much different, since it is obtriangular (figuras 11, 16-17) to slightly ovate (figuras 1, 3, 10). Other striking characteristics of *P. multifacies* sp. nov. are: (1) its comparatively small cell dimensions, (2) the very rapid locomotion, (3) the two centrally located concentric paramylum granules, and a third one eccentric, posterior or laterally placed regarding the central ones, (4) some specimens show a dorsal wing-like expansion next to the caudal process (figuras 5, 7, 24), and (5) the kind of cell displacement turning ventrally one full turn round itself (figuras 9, 27) and also rotating round its longitudinal axis.

Ecological remarks

A somewhat large number of individual specimens of the diatoms *Aulacoseira granulata* (Ehrenberg) Simonsen and *Aulacoseira ambigua* (Grunow) Simonsen and of the dinoflagellate *Peridinium gatunense* Nygaard were observed in every preparation from Lago da Ponte analyzed along the entire study period.

The pond is a very shallow system, station 1 being a little deeper (maximum depth 1 m, average depth 0.54 m) than station 2 (maximum depth 0.3 m, average depth 0.22 m). Individual specimens of *P. multifacies* were collected from depths ranging from 0.13 m to 0.35 m, *i.e.* always from very shallow totally transparent environments.

Phacus multifacies specimens were found living in acidic waters (pH 5.9-6.1), under a somewhat wide range of electric conductivity (90.6-154 $\mu\text{S cm}^{-1}$), dissolved organic matter (8.4-11 mg L^{-1}), dissolved oxygen (4.7-8.5 mg L^{-1}), percentage of dissolved oxygen (50.4-99%), water temperature (14.5-27.5 °C), and air temperature (18-28.5 °C) values. The species can be considered eurythermal due to its tolerance to a temperature amplitude greater than 10 °C.

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