

SCIENTIFIC COMMUNICATION

***Contraecaecum rudolphii* Hartwich (Nematoda, Anisakidae) from the Neotropical Cormorant, *Phalacrocorax brasilianus* (Gmelin) (Aves, Phalacrocoracidae) in southern Brazil<sup>1</sup>**

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**ABSTRACT.** The present report is part of a larger study on the helminth fauna of Neotropical Cormorants, *Phalacrocorax brasilianus* (Gmelin, 1789) in Brazil, particularly, in the southernmost State of Rio Grande do Sul. The nematodes which were found loose in the proventriculus/ventriculus or in groups of adults of different ages and of L<sub>3</sub> and L<sub>4</sub> larval stages, forming eosinophilic granulomas had a prevalence of 100% in 47 Neotropical cormorants from Lago Guaíba, Municipality of Guaíba. The morphology of the labia/interlabia, the distribution pattern of the caudal papillae in males examined under scanning electron microscopy (SEM), and the tips of the spicules allowed determination of the species as *Contraecaecum rudolphii* Hartwich, 1964 *sensu lato* (s. l.). This is the first record of *C. rudolphii* in southern Brazil.

**KEY WORDS.** Eosinophilic granulomas; Lago Guaíba; Rio Grande do Sul; SEM; taxonomy.

**RESUMO.** *Contraecaecum rudolphii* Hartwich (Nematoda, Anisakidae) parasita de biguás (cormorões Neotropicais, *Phalacrocorax brasilianus* (Gmelin) (Aves, Phalacrocoracidae) na região Sul do Brasil. O presente trabalho é parte de um estudo maior sobre a helmintofauna dos cormorões Neotropicais conhecidos no Brasil como biguás, *Phalacrocorax brasilianus* (Gmelin, 1789), particularmente, no Estado do Rio Grande do Sul. Os nematóides que foram encontrados soltos no proventrículo/ventrículo ou em grupos compostos por adultos de várias idades e larvas de vários estágios (L<sub>3</sub> e L<sub>4</sub>), formando granulomas eosinofílicos tiveram prevalência de 100% em 47 biguás do Lago Guaíba, Município de Guaíba. A morfologia dos lábios e dos interlábios, o padrão de distribuição das papilas caudais em machos examinados com microscópio eletrônico de varredura (MEV) e as extremidades dos espiculos permitiram a determinação da espécie como *Contraecaecum rudolphii* Hartwich, 1964 *sensu lato* (s. l.). Este é o primeiro registro de *C. rudolphii* na região Sul do Brasil.

**PALAVRAS-CHAVE.** Granulomas eosinofílicos; Lago Guaíba; MEV; Rio Grande do Sul; taxonomia.

HARTWICH (1964) revised *Contraecaecum* Railliet & Henry, 1912, presenting a complete list of synonyms and hosts for five species he considered as belonging to the genus. That author used the labia/interlabia internal and external morphology, the distribution of distal papillae, and the morphology of the spicules' tip to characterize these species as he had done earlier (HARTWICH 1957), when nomenclatorial alterations on the systematics of the Ascaridoidea were introduced. The author stated that *Ascaris microcephala* Rudolphi, 1809, later becoming *Contraecaecum microcephalum* (Rudolphi, 1809) Baylis, 1920, was morphologically similar to some specimens which Rudolphi had identified as *Ascaris spiculigera* Rudolphi, 1809,

and declared, based on page priority, *A. spiculigera* a junior synonym of *C. microcephalum*. Other specimens also studied by Rudolphi at that time were identified as *A. spiculigera*, although belonged to a new species (still unnamed) for which the new name *Contraecaecum rudolphii* Hartwich, 1964, was proposed.

FAGERHOLM (1988) discussed the pattern distribution of the caudal papillae, based on SEM, seen in several species of *Contraecaecum* parasites of seals at different localities in high latitudes of the northern and southern hemispheres. That author suggested a special nomenclature for the caudal papillae, including information on *C. rudolphii*. Later, FAGERHOLM (1991), considered the importance of studying the caudal papillae in a broader

sense, *i.e.*, within the superfamily Ascaridoidea. Other morphological studies with SEM were conducted by BARUŠ *et al.* (2000), showing the labia and interlabia morphology as well as the distribution of the caudal papillae of *C. rudolphii* and by ABOLLO *et al.* (2001) who characterized the male posterior region and the caudal papillae distribution in specimens collected from the European Shag *Phalacrocorax aristotelis* (Linnaeus, 1761).

The final hosts of the species of *Contraecum* are fish-eating birds (mostly Pelecaniformes) associated with fresh and marine waters and seals all over the world (Baruš *et al.* 2000). The main final hosts of *C. rudolphii* are birds of the genus *Phalacrocorax* Brisson, 1760 (ANDERSON 1992). THRELFALL (1982) recorded a concurrent infection by *C. spiculigerum* (= *C. rudolphii*) and *Contraecum multipapilatum* (Rudolphi, 1819) Baylis, 1920 in the Doubled-crested Cormorant, *Phalacrocorax auritus* (Lesson, 1831) while ABOLLO *et al.* (2001) recorded concurrent infections by *C. rudolphii* and *Contraecum septentrionale* Kreis, 1955 in the proventriculus of the European shag.

The Neotropical Cormorant, *Phalacrocorax brasilianus* (Gmelin, 1789), occurs from Tierra del Fuego, Patagonia, Argentina to coastal Texas, USA, the northernmost extreme of its known geographical distribution (DEL HOYO *et al.* 1992, *apud* FEDYNICH *et al.* 1997). The resident populations of this bird have been the subject of helminthological studies in southern Chile by TORRES *et al.* (1981, 1982, 1983, 2000) and in the coastal Texas, USA by FEDYNICH *et al.* (1997), who compared the helminth community structure and pattern in sympatric populations of double-crested and Neotropical cormorants.

VICENTE *et al.* (1995a) published valuable information about the nematodes of Brazilian birds, mostly based on specimens deposited in the "Coleção Helminológica do Instituto Oswaldo Cruz (CHIOC)", from the early 1920's to the late 1960's. VICENTE *et al.* (1995b) listed the nematodes of Brazilian ciconiiform birds, establishing new records, and later, VICENTE *et al.* (1996), made a brief description of four specimens of *C. spiculigerum* (= *C. rudolphii*) collected from *P. brasilianus* and the "anhinga", *Anhinga anhinga* (Linnaeus, 1758), from the States of Mato Grosso do Sul and Rio de Janeiro, which were deposited in the CHIOC. The present study is the first to identify the species of *Contraecum* from *P. brasilianus* in Lago Guaíba, Municipality of Guaíba, Rio Grande do Sul, southern Brazil.

Forty-seven Neotropical cormorants, *P. brasilianus* were collected from Lago Guaíba, Municipality of Guaíba, Rio Grande do Sul, from 1999 to 2003, in accordance with the permit numbers 232/1999 and 064/2002, from Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis (IBAMA). Cormorants were frozen at -10°C, as soon as possible, after the catch. Necropsies were performed in the Laboratório de Helminologia, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Brazil. The nematodes were processed according to AMATO *et al.* (1991). Helminths were collected, counted, and cleaned in saline solution 0.85%. Later were fixed in AFA and preserved in 70%GL ethanol. For light microscopy (LM) some specimens

were examined as temporary whole mounts in lactophenol (HUMASON 1972). For SEM, the nematodes with sectioned spicules were critical point dried, coated with carbon and gold, and examined with a Jeol (JSM-6060) scanning electron microscope. Measurements are in millimeters (mm) unless otherwise indicated, ranges are followed (between parentheses) by the mean  $\pm$  the standard deviation values, and the number of specimens measured for a given character (when different than the total number of specimens measured). Morphological parameters follow HARTWICH (1964). Different developmental stages were identified according to HARTWICH (1964) and HUIZINGA (1966). The fourth larval stage (L<sub>4</sub>) and immature adults were identified according to TORRES *et al.* (2000). The nomenclature for the pattern of male caudal papillae follows FAGERHOLM (1988). Ecological terms such as prevalence, intensity of infection, and mean intensity of infection follow MARGOLIS *et al.* (1982). Voucher specimens were deposited (in ethanol 70%GL) in the CHIOC, Rio de Janeiro, Rio de Janeiro, Brazil.

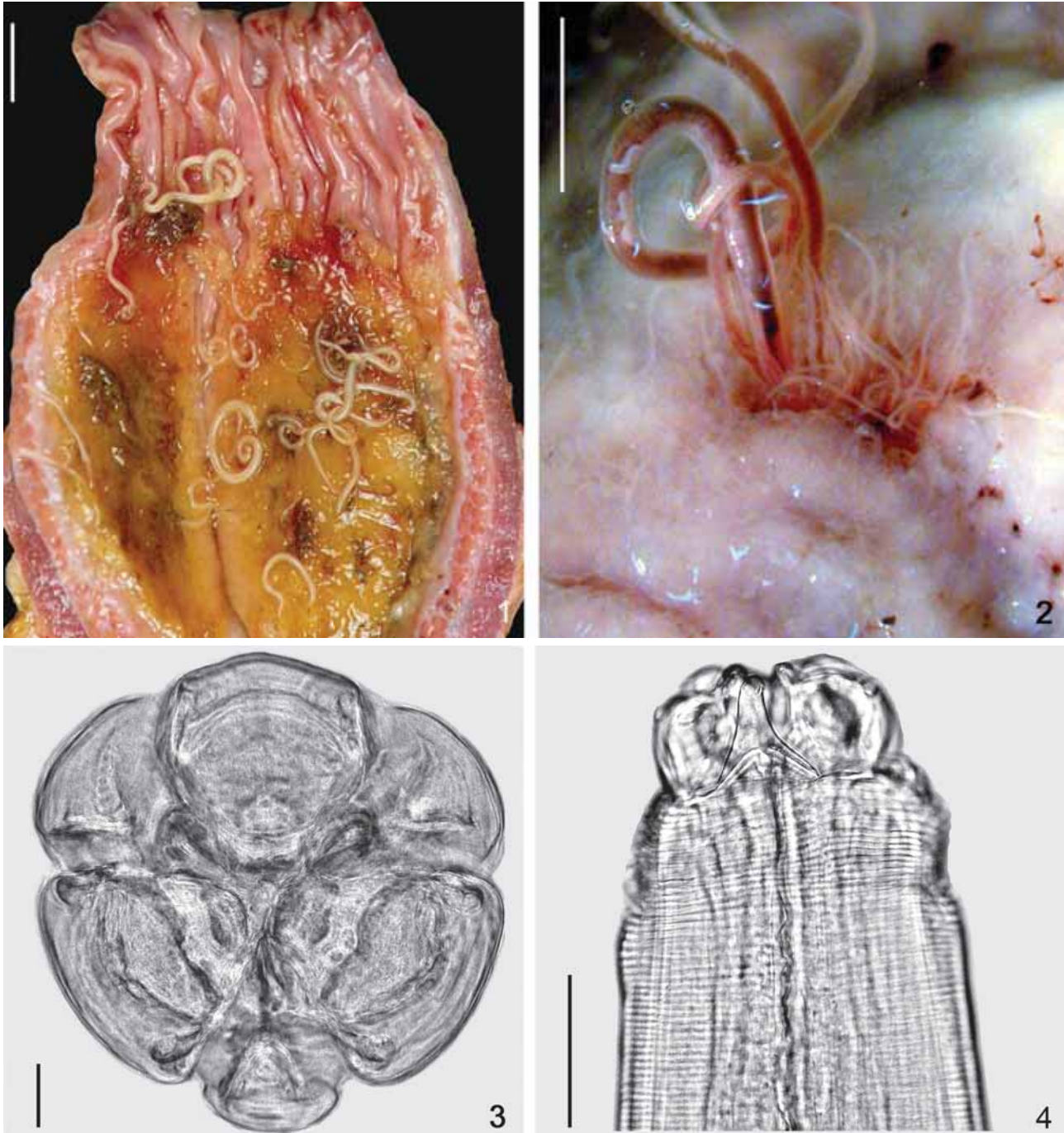
### *Contraecum rudolphii* Hartwich, 1964

#### Figs 1-9

Description: based on 105 adult specimens (30 males and 30 females mounted in lactophenol and measured; five specimens mounted *en face* as well as four male tails mounted in lactophenol; and 46 specimens mounted on stubs for SEM).

General: nematodes of different ages and larval stages were found concentrated in the proventriculus/ventriculus (Fig. 1), forming ulcerated eosinophilic granulomas (Fig. 2) and feeding on blood. Three labia, usually hexagonal, without denticerous ridges (Figs 3, 5 and 6); dorsal labium with 2 ovate papillae (Figs 5 and 6), each formed by the fusion of the two original papillae; 2 ventrolateral labia, each with 1 lateral, ovate papilla (Figs 3 and 5), also formed by the fusion of the two original papillae and 1 amphidial pore (Fig. 5 – arrows) and two auriculae (Fig. 6 – head arrows); interlabia well developed (Figs 3-5), with bifid distal ends (Fig. 7). Excretory pore opening bellow ventral interlabium. Esophagus with small, globular ventricle and a posterior ventricular appendix. Intestinal cecum directed anteriorly.

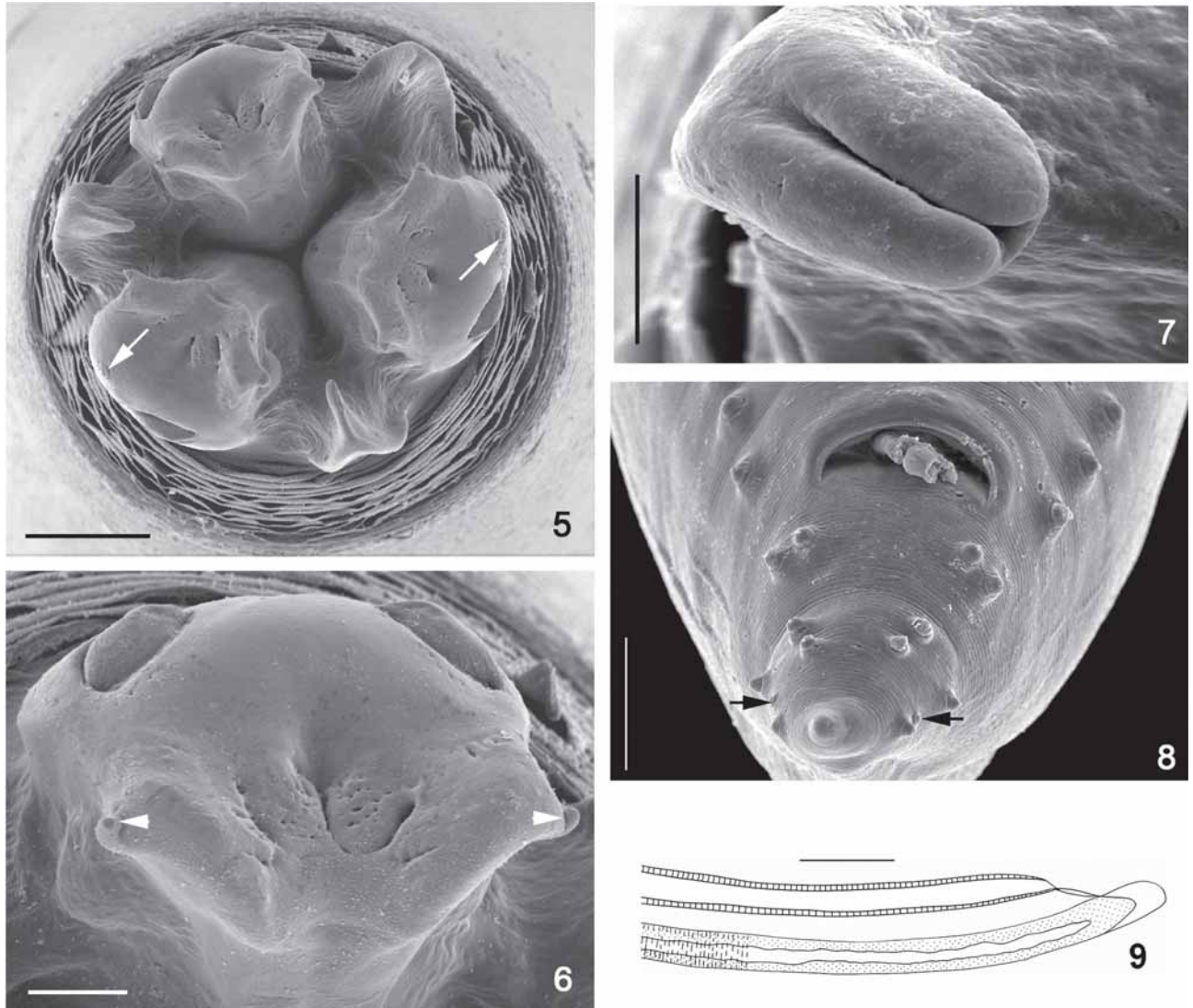
Males (30 specimens): body 18-31 (25  $\pm$  3.4) long, 306-598  $\mu$ m (481  $\pm$  7  $\mu$ m) in maximum width. Muscular esophagus 2.4-3.8 (3.1  $\pm$  0.5) long; intestinal cecum 2.1-2.9 (2.4  $\pm$  0.3) long; ventricular appendix 0.8-1.2 (1.0  $\pm$  0.1) long. Spicules similar, almost equal (Fig. 9); right spicule 4.5-7.5 (6.2  $\pm$  0.9) long, left spicule 5.9-8.2 (7.1  $\pm$  0.8) long; cloacal opening at 140-235  $\mu$ m (205  $\pm$  22  $\mu$ m) from posterior end. Tail conic, with more than 30 pairs of proximal papillae, 1 or 2 pairs of ad-cloacal papillae (Fig. 8), 7 pairs of distal papillae, the first 2 pairs side by side; 2 ventrolateral and 2 dorso-lateral pairs (Fig. 8 – arrows). Phasmidial pores on top of smaller papillae, between ventrolateral papillae (Fig. 8). Body length/body maximum width ratio 47.9-68.6:1 (52.9  $\pm$  3.3); body length/esophagus length ratio 7.1-10.9:1 (8.1  $\pm$  0.7); body length/caudal length



Figures 1-4. (1-2) Neotropical cormorant proventriculus infected with *Contracaecum rudolphii*: (1) general view of loose individuals of different sizes, showing continuous recruitment, bar = 10 mm; (2) nematodes of different sizes attached to proventriculus mucosa, showing that they ingest blood and that there is continuous recruitment, bar = 5 mm; (3-4) anterior region of *Contracaecum rudolphii*, viewed with light microscopy: (3) *en face* view of anterior end, bar = 20  $\mu$ m; (4) ventral view, showing ventral interlabium, bar = 100  $\mu$ m.

ratio 91.3-145.5:1 ( $122.7 \pm 14.6$ ); esophagus length/intestinal cecum length ratio 0.9-1.6:1 ( $1.3 \pm 0.1$ ); esophagus length/ven-

tricular appendix length ratio 2.8-3.6:1 ( $3.2 \pm 0.2$ ); body length/mean length of spicules ratio 3.1-5.4:1 ( $3.8 \pm 0.4$ ).



Figures 5-9. *Contracaecum rudolphii*. (5-8) Viewed with SEM: (5) *en face* view of anterior end, showing amphidial pores (arrows), bar = 30  $\mu$ m; (6) dorsal labium with two auriculae (head arrows), bar = 10  $\mu$ m; (7) interlabium with bifid tip, bar = 10  $\mu$ m; (8) male tail, showing cloacal opening with sectioned spicules, caudal papillae, and phasmidial pores on top of papillae (arrows), bar = 50  $\mu$ m; (9) diagram showing the tip of a spicule, according to HARTWICH (1964), bar = 20  $\mu$ m.

Females (30 specimens): body 23-52 ( $41.8 \pm 7.5$ ) long, 0.5-1.1 ( $0.8 \pm 0.1$ ) wide. Muscular esophagus 2.4-5.4 ( $4.2 \pm 0.8$ ) long; intestinal cecum 1.6-3.6 ( $2.9 \pm 0.6$ ) long; ventricular appendix 0.6-1.5 ( $1.2 \pm 0.2$ ) long; anus 0.2-0.6 ( $0.4 \pm 0.1$ ) from posterior end. Vulva 9.7-21.3 ( $15.2 \pm 2.5$ ) from anterior end. Eggs 99-106  $\mu$ m ( $105 \pm 7 \mu$ m) long, 83-92  $\mu$ m ( $91 \pm 8 \mu$ m) wide. Phasmid apertures lateral. Body length/body maximum width ratio 36.6-66.5:1 ( $53.2 \pm 6.1$ ); body length/esophagus length ratio 8.7-13.2:1 ( $9.9 \pm 0.8$ ); body length/caudal length ratio 74.9-168.3:1 ( $101.8 \pm 21.0$ ); esophagus length/intestinal caecum length ratio 1.3-1.6:1 ( $1.5 \pm 0.1$ ); esophagus length/ven-

tricular appendix length ratio 3.2-4.3:1 ( $3.7 \pm 0.3$ ). Vulva from anterior extremity x 100/body length ratio 29.7-46.5:1 ( $36.7 \pm 3.6$ ).

Host. Neotropical cormorant ("biguá"), *Phalacrocorax brasilianus* (Gmelin, 1789).

Site of infection. Proventriculus/ventriculus.

Locality. Lago Guaíba, Municipality of Guaíba, Rio Grande do Sul, Brazil (30°00'S, 51°15'W).

Prevalence. 100%.

Mean intensity of infection. 97.7 helminths/host.

Range of infection. 25-403 helminths/host.

Voucher specimens deposited. CHIOC numbers 35.488 and 35.489 (in ethanol 70%GL).

Remarks. Generally, measurements and morphometrical ratios in our specimens were larger than those given by HARTWICH (1964), although these differences might be related to the fact that we only measured adult specimens, while Hartwich did not say if the measurements presented referred only to adult specimens. The distal end of the spicules (Fig. 9) and the number and position of caudal papillae (Fig. 8) when compared with the original description, confirm the identification of this species.

HARTWICH (1964) indicated the holotype, paratypes, type host, and type locality for *C. rudolphii*, stating (pg. 32): "Der Holotypus befindet sich in der Helminthensammlung des Instituts für Spezielle Zoologie und Zoologischen Museums der Humboldt-Universität zu Berlin unter der Katalog-Nr. 441a, die Paratypen unter Nr. 441b" [*sic*].

VICENTE *et al.* (1996) published morphometrical data and illustrations of specific diagnostic characters of *C. spiculigerum* (= *C. rudolphii*) allowing comparison with the specimens of the present report, which are larger. The illustrations provided by these authors of the *en face* anterior end cannot be accepted as representative of the species, as well as the indication of the presence of two labial papillae in each labium. HARTWICH (1964), in the original description of the species, and anteriorly HARTWICH (1957), when proposing the genus, mentioned the presence of two pairs of papillae in the dorsal labium, resulting from the fusion of the two original papillae. VICENTE *et al.* (1996) have also indicated the presence of five pairs of distal papillae, when the generic diagnosis indicates the presence of seven pairs.

Another discrepancy between Hartwich's description and that of VICENTE *et al.* (1996) are the egg sizes: 59-73  $\mu\text{m}$  long by 41-59  $\mu\text{m}$  wide and 50  $\mu\text{m}$  long by 40  $\mu\text{m}$  wide, respectively. The size of eggs in the specimens of *C. rudolphii* in the present work is larger than what has been indicated by these authors, and also have the phasmidial openings on top of the penultimate pair of distal papillae (Fig. 8 - arrows), as has been indicated by FAGERHOLM (1988) and ABOLLO *et al.* (2001). The shape of the labia and interlabia, as well as the papillae distribution on the male tails and the bifurcation of the interlabial extremities, agree with the illustrations provided by BARUŠ *et al.* (2000).

HARTWICH (1964) listed the ardeid "*Casmerodius albus egretta* (Linnaeus, 1758) (Syn.: *Ardea leuce* Bryant). Brasilien (Rio Grande do Sul)" [*sic*], as one of the hosts for *C. microcephalum*, giving no additional information (author, collecting date, exact locality of capture, etc.), but indicating the deposit number in the collection: "Zool. Museum Berlin (N° 1071)" [*sic*]. This is the only previous record of any species of *Contraecum* in southern Brazil, but cannot be confirmed at the present time.

LI *et al.* (2005) presented genetic evidence for the existence of sibling species within the *C. rudolphii* complex (formed by *C. rudolphii* A and *C. rudolphii* B) collected from the Large Black Cormorant, *Phalacrocorax carbo sinensis* Blumenbach,

1798, in northeastern and central Italy, based on the first (ITS-1) and the second (ITS-2) internal transcribed spacers of ribosomal DNA (rDNA). As specimens of *C. rudolphii* from the proventriculus/ventriculus of *P. brasiliensis* from Lago Guaíba still have to undergo examination at the molecular level to detect the eventual existence of sibling species the present work is the first to document the presence of *C. rudolphii* (s. l.) in *P. brasiliensis* in southern Brazil.

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