

## ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

### A NEW SPECIES OF *PHYSALOPTERA* (NEMATODA: PHYSALOPTERIDAE) FROM *CERRADOMYS SUBFLAVUS* (RODENTIA: SIGMODONTINAE) IN THE CERRADO BIOME, BRAZIL

### UNA NUEVA ESPECIE DE *PHYSALOPTERA* (NEMATODA: PHYSALOPTEROIDEA) DE *CERRADOMYS SUBFLAVUS* (RODENTIA: SIGMODONTINAE) EN EL BIOMA CERRADO, BRAZIL

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Neotropical Helminthology, 2015, 9(2), jul-dec: 301-312.

## ABSTRACT

*Physaloptera galvai* n.sp. is described from the stomach of *Cerradomys subflavus* (Rodentia: Sigmodontinae) collected in Serra da Canastra National Park (São Roque de Minas municipally, Minas Gerais state, Brazil). The genus *Physaloptera* includes 13 species infecting rodents worldwide. Among these, five occur in the Americas. The main characteristics of the new species are size, shape and relation of spicule length to total body length, pre and postcloacal papillae equidistant in males, and uterus with four to five branches in females. In relation to the *Physaloptera* spp. that parasitize rodents in the Americas, the new species can be differentiated from congeners as follows: *Physaloptera bispiculata*, *Physaloptera murisbrasilensis*, *Physaloptera calnuensis*, *Physaloptera longispicula*, and *Physaloptera hispida* because it is the only one to have a short spicule and to present first and second pairs of papillae asymmetrically displaced (left one anterior to right one) on male and tetra and pentadelph on female, therefore representing a new species for the genus. Thus, the specimens of nematodes parasites from stomach of the *C. subflavus* represent a new species of Physalopteridae family named *P. galvai* n.sp.

**Key words:** nematode - rodent - taxonomic morphology - light microscopy - scanning electron microscopy

## RESUMEN

*Physaloptera galvaoi* n.sp. fue registrada parasitando en el estómago de *Cerradomys subflavus* (Rodentia: Sigmodontinae) capturados en el Parque Nacional Serra da Canastra (São Roque de Minas, Minas Gerais, Brasil). El género *Physaloptera* tiene 13 especies que parasitan los roedores en todo el mundo. Entre ellos, cinco fueron registrados en las Américas. Las principales características de la nueva especie son el tamaño, la forma de la espícula y la relación de longitud de la espícula con la longitud total del cuerpo, las papilas pre y pos cloacal equidistantes en los espécimen masculinos, y el útero con cuatro hasta cinco ramas en las hembras. La nueva especie difiere de las otras especies del género *Physaloptera* registradas en América como *Physaloptera bispiculata*, *Physaloptera murisbrasiliensis*, *Physaloptera calnuensis*, *Physaloptera longispicula* y *Physaloptera hispida* por tener: (1) una espícula pequeña, (2) primero y segundo par de papilas asimétricamente desplazadas (la izquierda anterior a la derecha), (3) útero de cuatro hasta cinco ramas (tetra y pentadelpho). Estos caracteres morfológicos representan a *P. galvaoi* como una nueva especie para el género. Por lo tanto, los especímenes de nemátodos parásitos de estómago del hospedador *C. subflavus* representan una nueva especie de la familia Physalopteridae llamado *P. galvaoi* n.sp.

**Palabras clave:** microscopía de luz - microscopía electrónica de barrido - nematode - roedor - taxonomía morfológica.

## INTRODUCCIÓN

Nematodes of the genus *Physaloptera* Rudolphi, 1819 are parasites in the stomach of reptiles, birds, mammals and occasionally amphibians. They infect both wild animals and pets, such as dogs and cats (Ortlepp, 1922; Chabaud, 1975).

At present, 100 species of *Physaloptera* are known (Pereira *et al.*, 2012). Among these, 13 have been described infecting rodents worldwide, including four species on South America: *P. bispiculata*, Vaz and Pereira, 1935; *P. murisbrasiliensis* Diesing, 1860; *P. longispicula* Quentin, 1968 (Vicente *et al.*, 1997); and *P. calnuensis* Sutton, 1989, with only one in North America, *P. hispida* Schell, 1950.

The rice rat, *Cerradomys subflavus* (Wagner, 1842) (syn. *Oryzomys subflavus*) has terrestrial habits and feeds on seeds, leaves and arthropods (Souza *et al.*, 2004). This rodent

was originally described in Lagoa Santa, Minas Gerais and also occurs in the Brazilian states of Góias, São Paulo and Bahia (Langguth & Bonvicino, 2002; Percequillo *et al.*, 2008).

The vegetation of Serra da Canastra National Park is characterized as an ecotone of Atlantic Forest and cerrado biomes, with predominance of high-altitude fields that harbor numerous species of cerrado (savanna) fauna and flora. Most of the Park consists of meadow interspersed with forest, and the soil is acidic and poorly drained. Grasses, sedges and pipeworts predominate. The dry herbaceous fields mainly contain grasses, while the shrubby fields contain grasses and shrubs or small trees that are widely spaced (Romero & Nakajima, 1999).

In the present study, a new species of *Physaloptera* (Spirurida: Physalopterinae) is described found parasitizing the stomach of *C. subflavus* in São Roque de Minas municipally of Minas Gerais state.

## MATERIAL AND METHODS

Nematodes were collected from the stomach of a naturally infected *C. subflavus* in the Serra da Canastra National Park (20°13'28.30" S; 46°30'39.20" W), municipality of São Roque de Minas, state of Minas Gerais, Brazil. Collection was carried out in May 2010, using a Tomahawk® trap (model 201; 16 x 5 x 5 inches or 40.6 x 12.7 x 12.7 cm) and a Sherman® trap (model XLK; 3 x 3.75 x 12 inches or 7.6 x 9.5 x 30.5 cm). This study was approved by the ethics committee of Oswaldo Cruz Foundation/Fiocruz (CEUA L-0015/07). Capture and sampling of small mammals was authorized by Chico Mendes Institute for Conservation of Biodiversity (ICMBIO/SISBIO, license number 18635-1). For euthanasia, the animals were heavily anesthetized with an intramuscular injection of thiopental sodium, followed by intracardiac administration of potassium chloride, under the supervision of a licensed veterinarian. Parasites were washed in saline and had their anterior and posterior ends cut and fixed in hot AFA (2% glacial acetic acid, 3% formaldehyde, and 95% of 70° ethanol) for morphological identification. Drawings were made using a drawing tube attached to a Zeiss standard microscope. Measurements are in millimeters unless otherwise stated for holotype and allotype. The range (paratypes) in brackets and mean in parentheses. Type species of *Physaloptera murisbrasiliensis* (CHIOC 9493) and representatives specimens of *Physaloptera bispiculata* (CHIOC 36798) collected from *Nectomys squamipes* Brants, 1827, municipality of Sumidouro, Rio de Janeiro deposited in the Helminth Collection of the Oswaldo Cruz Institute (CHIOC), were used for study.

The nematodes were clarified in 80% phenol and mounted on temporary slides. Morphological analyses were conducted using an Olympus BX-51 light microscope and

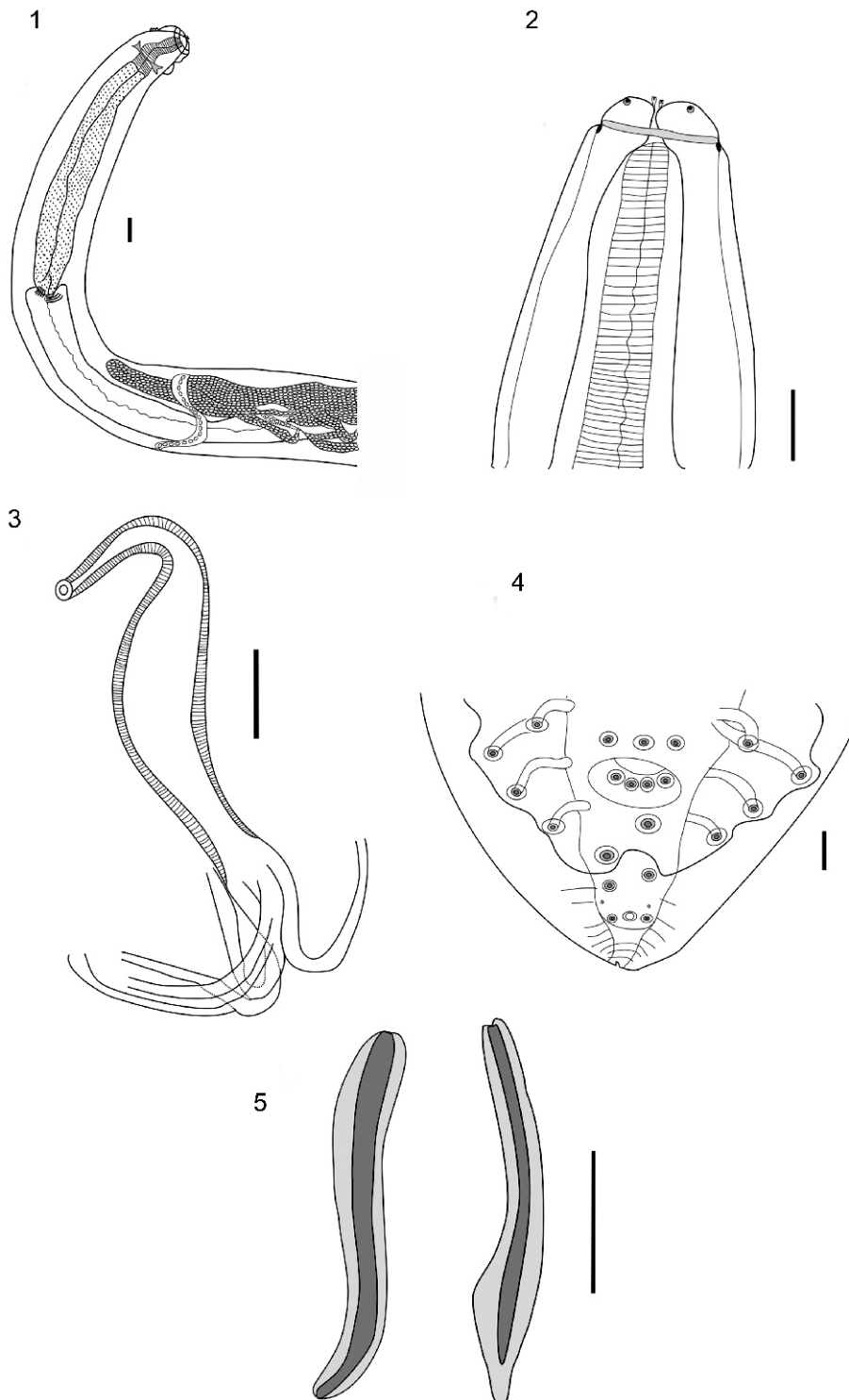
images were captured using an Olympus DP-12 digital camera.

Five male and five female nematode specimens were separate for SEM. These specimens were washed in 0.1 M Na-cacodylate buffer, pH 7.2, post-fixed in 1% OsO<sub>4</sub> and 0.8% K<sub>3</sub>Fe(CN)<sub>6</sub>, dehydrated in graded ethanol (30-100%) for 2 h, and dried by the critical point method with CO<sub>2</sub> (CPD 030, Balzers, Switzerland). The samples were mounted on aluminum stubs, coated with a 20-nm layer of gold and examined with a Jeol JSM 6390LV scanning electron microscope (operating at 15kV) at the Rudolf Barth Electron Microscopy Platform of Oswaldo Cruz Institute.

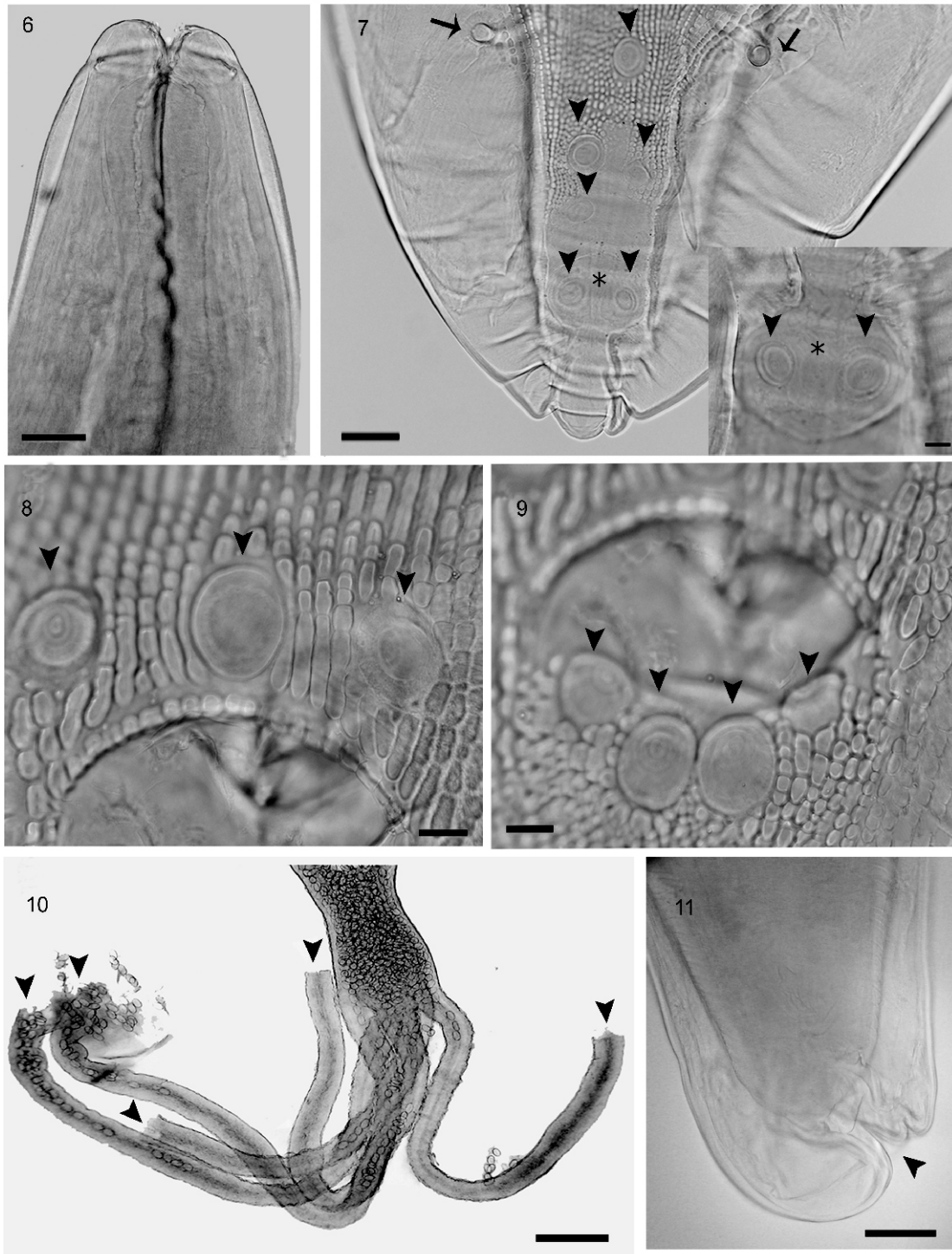
## RESULTS

*General:* Body robust, elongated with fine cuticular striations, tapering gradually at the anterior end. Females more robust than males. Cuticle in cephalic region reflecting to posterior margins of pseudolabia, forming cephalic collar (Figs. 2, 6 and 12). Oral opening with two large lateral, semicircular pseudolabia, each bearing three internal lateral teeth, forming a tripartite structure and single large external lateral triangulate tooth with cuticular fold (Figs. 12, 13 and 15). Tripartite internal tooth composed of unequal tips, two rounded and one pointed triangular, with a pore on internal surface (Fig. 15). Two pairs of small teeth in the external lateral cuticular fold on each side, totaling four small teeth. Each lip has one pair of papillae in depression and one amphid between them (Figs. 13 and 14). Three well-delimited porous areas present on each lip (Figs. 12 and 13). Cuticle with transverse striations (Fig. 13). Esophagus long, divided into anterior muscular and posterior glandular parts (Figs. 1, 2 and 6). Nerve ring encircling final portion of muscular esophagus (Fig. 1).

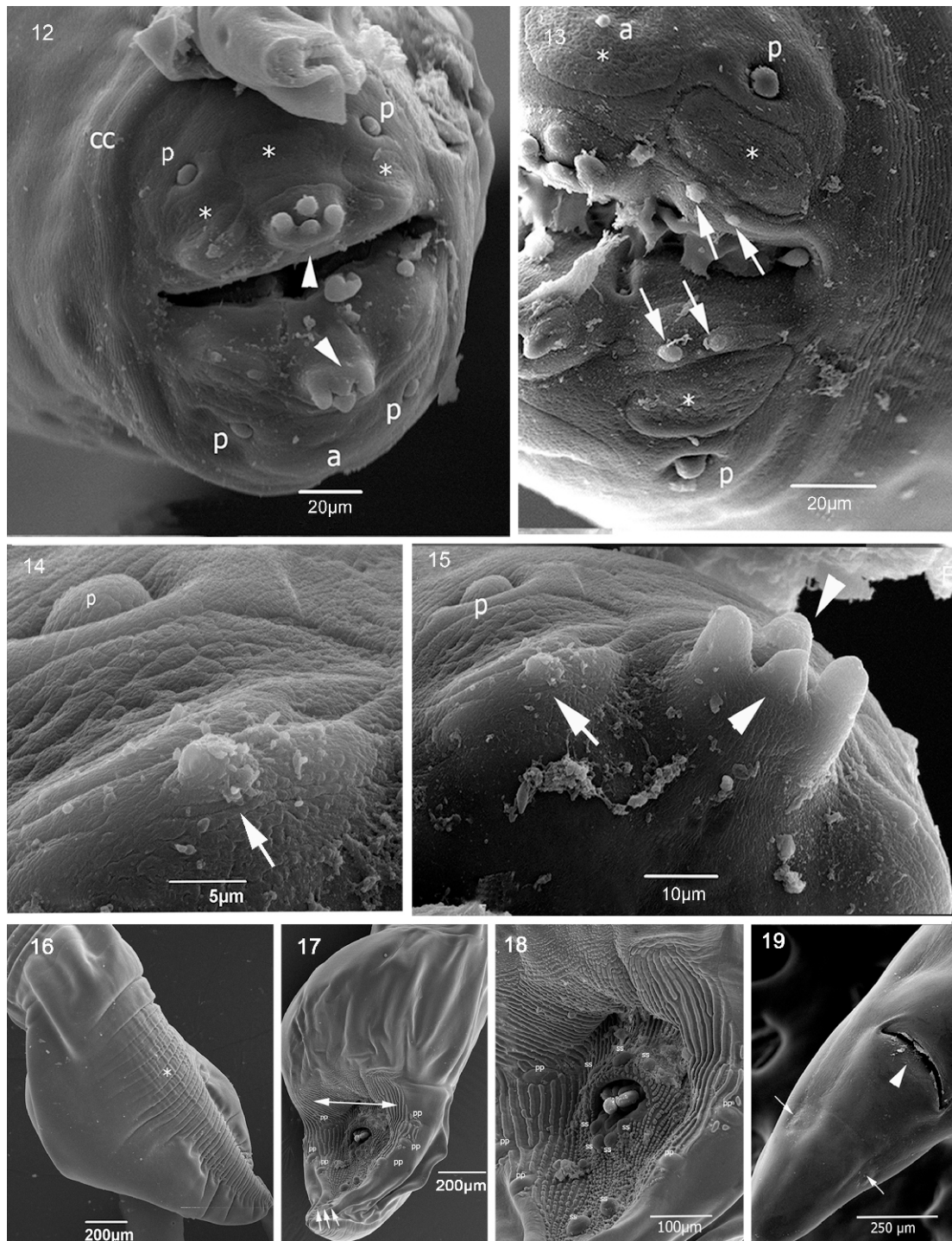
*Male (holotype and six paratypes):* 12.4 [10-



**Figures 1-5.** *Physaloptera galvai* n.sp. (1) Female anterior end showing muscular and glandular esophagus, nerve ring, vulva opening. Scale bar: 100  $\mu$ m. (2) Male anterior end showing tripartite tooth, cephalic papillae and cephalic collar. Scale bar: 100  $\mu$ m. (3) Uterus with five uterine branches. Scale bar: 500  $\mu$ m. (4) Male posterior end with four pairs of pedunculated papillae, thirteen sessile papillae and one median protuberance-like papilla. Scale bar: 100  $\mu$ m. (5) Left (L) and right (R) spicule. Scale bar: 100 $\mu$ m.



**Figures 6-11.** Light microscopy *Physaloptera galvai* n.sp. (6) Male anterior end and dorsal view. (7) Male posterior end, ventral view showing pedunculated papillae (arrow) and sessile papillae (arrowhead); Detail of fifth pair sessile papilla (arrowhead) and median protuberance-like papilla (\*); (8) Ventral view of the precloacal papillae. Scale bar: 500 µm; (9) Ventral view of two pairs postcloacal papillae (arrowhead). Scale bar: 100 µm; (10) Uterus with five uterine branches (arrowhead). (11) Female posterior end showing anus (arrowhead). Scale bar: 100 µm.



**Figures 12-19.** Scanning electron microscopy *Physaloptera galvaoui* n. sp. (12). Female anterior end showing pseudolabia with cephalic papillae (p), tripartite and small teeth (arrowhead), cervical region (cc), amphid (a) and porous areas (•). (13). Detail showing two pairs of lateral small teeth on pseudolabia (arrows). (14). Detail of small tooth (arrow) and cephalic papilla (p). (15). Detail tripartite teeth (arrowhead), small tooth (arrow) and cephalic papilla (p). (16). Male posterior end in dorsal view. (17). Male posterior end in ventral view showing two lateroventral folds (double arrow). (18). Male posterior end in ventral view, pairs pedunculated papillae (pp), sessile papillae (ss), cloacal opening and one median protuberance-like papilla (arrow). (19). Female posterior end, showing the anal opening (arrowhead) and phasmids (arrow).

23 (15.5)] mm long, 0.64 [0.8-0.9 (0.8)] mm wide at mid-body. Muscular and glandular esophagus 0.5 [0.46-0.58 (0.5)] and 2.62 [3.0-3.74 (3.4)] long, respectively. Nerve ring and excretory pore 0.3 [0.2-0.4 (0.4)] and 0.5 [0.52-0.95 (0.8)] from the anterior end, respectively (Fig. 1; Table 1). Male posterior end with caudal fold not tapered at the end (Figs. 4 and 7). Cloacal opening is crescent shaped (Figs. 8 and 9). Twenty-one caudal papillae, four pedunculated externolateral papillae on each side of the caudal alae, three sessile precloacal papillae different in size, a single median papilla and one pair of papillae situated on the same transverse line (Figs. 4, 7 and 8), and two pairs of sessile papillae slightly posterior to cloacal opening (Figs. 4 and 9). Three pairs of postcloacal papillae equidistant to each other: first and second pairs asymmetrically displaced (left one anterior to right one), third pair symmetrical with a median protuberance-like papilla (Figs. 4, 7, 17 and 18). Male tail 0.47 [0.28-0.61 (0.47)], Posterior ventral end ornate with longitudinal ridges, lateral region finely striated and central region rough. Smooth dorsal surface caudal alae (Fig. 16). Spicules are sub-equal and dissimilar. Left spicule lanceolate, 0.26 [0.23-0.28 (0.26)] long, right spicule stout and slightly curved at tip, 0.21 [0.19-0.23 (0.21)] long, representing 1.57% and 1.27% of total body length (SpL/BL), respectively (Fig. 5, Table 1).

*Female (allotype and eight paratypes):* 11.58 [16.4-31.3 (23.28)] mm long and 1.06 [1.10-3.18 (1.44)] mm wide at mid-body. Muscular and glandular esophagus 0.49 [0.42-0.71 (0.56)] and 1.44 [1.27-4.14 (2.7)], respectively. Nerve ring and excretory pore 0.20 [0.20-0.28 (0.19)] and 0.7 [0.68-1.66 (1.17)] from the anterior end, respectively (Figs. 1; Table 2). Uterus varied from four to five uterine branches (Figs. 3 and 10). Vulva opening 9.0 [6.42-11.14 (8.78)] from the anterior end (Fig. 1). Rounded lateral phasmids between the anus and tip of conical

tail (Fig. 19).). Eggs elliptical 0.04 [0.04-0.05 (0.05)] long and 0.03 [0.02-0.03 (0.03)] wide. Anus opening 0.5 [0.43-0.50 (0.46)] from the posterior end (Figs. 11 and 19; Table 2).

#### Taxonomic summary

*Type host:* *Cerradomys subflavus* (Wagner, 1842) (Rodentia: Cricetidae).

*Site of infection:* Stomach.

*Type locality:* São Roque de Minas municipality, Serra da Canastra National Park, Minas Gerais, Brazil (20°13'28.30" S; 46°30'39.20" W).

*Prevalence of infection:* 43% (3 rodents infected/7 rodents collected)

*Specimens deposited:* Holotype male (CHIOC no. 36758a) and allotype female (CHIOC no. 36758 b) were deposited in the Helminth Collection of the Oswaldo Cruz Institute, Rio de Janeiro, Brazil.

*Etymology:* New species is named in honor of Dr. Cléber Galvão Ferreira for his contributions to parasitology.

## DISCUSSION

The genus *Physaloptera* was created by Rudolphi (1819) having as type species *Physaloptera clausa* parasitizing *Erinaceus dealbatus* Swinhoe, 1870. Dujardin (1845) suppressed the genus *Physaloptera*, transferring its species to the genus *Spiroptera*. Later, Diesing (1851) re-established *Physaloptera* genus and included two new species. Seurat (1914) highlighted the taxonomic importance of uterus number within the genus *Physaloptera* and reorganized species groups according to the presence of two or four uterus (Ortlepp, 1922).

Travassos (1920) maintained within *Physaloptera* the species having up to two uterus and created the *Turgida* genus from the description of *T. torresi*, using as criterion the presence of 10 uterus, and transferred

**Table 1.** Morphological features and measurements, vertebrate host and locality of *Physaloptera* spp. males infecting rodents from Americas.

|                                     | <i>P. murisbrasiliensis</i><br>Diesing, 1860 | <i>P. bispiculata</i><br>Vaz & Pereira, 1935 | <i>P. hispida</i><br>Schell, 1950 | <i>P. longispicula</i><br>Quentin, 1968 | <i>P. caluensis</i><br>Suton, 1989 | <i>P. galvaoi</i> n. sp.<br>Present study |
|-------------------------------------|--|--|-----------------------------------|---|------------------------------------|---|
| Length                              | 22-28  | 25.0   | 30-42                             | 34.4                                    | 17.1                               | 10-23                                     |
| Width                               | 0.87-0.95                                    | 0.8  | 0.9-1.4                           | 1.4                                     | 0.61                               | 0.8-0.9                                   |
| Glandular esophagus                 | -  | 3.2  | 4.2-5.3                           | 5.3                                     | 2.99                               | 3.0-3.74                                  |
| Muscular esophagus                  | -  | 0.5  | 0.56-0.73                         | 0.6                                     | 0.33                               | 0.58-0.46                                 |
| Nerve ring*                         | -  | -  | 0.55-0.7                          | 0.5                                     | 0.34                               | 0.20-0.43                                 |
| Excretore pore                      | -  | -  | 0.87-1.2                          | 0.83                                    | 0.80                               | 0.52-0.95                                 |
| Deirids                             | -  | -  | 0.67-0.91                         | 0.7                                     | 0.95                               | -   |
| Tail**                              | -  | 0.87   | -                                 | 1.9                                     | 0.58                               | 0.28-0.61                                 |
| Right spicule                       | 0.40   | 0.40   | 0.39-0.55                         | 0.71                                    | 0.31                               | 0.19-0.23                                 |
| SpL/BL                              | 1.6%   | 1.6%   | 1.29%                             | 2.06%                                   | 1.81%                              | 1.57%                                     |
| Left spicule                        | 0.40   | 0.46   | 0.34-0.48                         | 0.85                                    | 0.42                               | 0.23-0.28                                 |
| SpL/BL                              | 1.6%   | 1.84%  | 1.13%                             | 2.47%                                   | 2.45%                              | 1.27%                                     |
| Caudal papillae δ                   | 22   | 21   | 21                                | 21                                      | 21                                 | 21  |
| protuberance-like papilla           | np   | p  | p                                 | np                                      | p                                  | p   |
| Host                                | <i>Mus brasiliensis</i>                      |  |                                   | <i>Thrichomys apereoides</i>            |                                    |   |
| (= <i>Holochilus brasiliensis</i> ) |  | <i>Nectomys squamipes</i>                    | <i>Sigmodon hispidus</i>          | (= <i>Cercomys cunicularius</i> )       | <i>Calomys laucha</i>              | <i>Cerrdomys subflavus</i>                |
| Site of infection                   | Stomach                                      | Stomach                                      | Stomach                           | Stomach                                 | Stomach                            | Stomach                                   |
| Locality                            | Brazil                                       | Brazil                                       | USA                               | Brazil                                  | Argentina                          | Brazil                                    |

p = present; np = not present by the authors; SpL/BL = relation spicule length by total body length; all measurements are in millimetres. \*Distance from the anterior end; \*\*Distance from the cloaca to the posterior end; Number.



Table 2. Morphological features and measurements, vertebrate host and locality of *Physaloptera* spp. females infecting rodents from Americas.

|                     | <i>P. murisbrasilienis</i><br>Diesing, 1860 | <i>P. bispiculata</i><br>Vaz & Pereira, 1935 | <i>P. hispida</i><br>Schell, 1950   | <i>P. calnuensis</i><br>Suton, 1989 | <i>P. galvaoi</i> n. sp.<br>Present study |
|---------------------|---|--|-------------------------------------|-------------------------------------|---|
| Length              | 35-43                                       | 27-55  | 53-64                               | 28.07                               | 9.3-1.42                                  |
| Width               | 1.1-1.75                                    | 1.0-1.9                                      | 1.9-2.0                             | 0.99                                | 1.10-3.18                                 |
| Glandular esophagus | -   | 3.8-5.4                                      | 5.0-7.0                             | 3.32                                | 1.27-4.14                                 |
| Muscular esophagus  | -   | 0.54-0.7                                     | 0.64-0.69                           | 0.39                                | 0.42-0.71                                 |
| Nerve ring*         | -   | -  | 0.71-0.74                           | 0.38                                | 0.20-0.28                                 |
| Excretore pore      | -   | 1.2  | 0.84-1.08                           | 0.78                                | 0.68-1.66                                 |
| Deirids             | -   | -  | 0.67-0.91                           | 0.77                                | -   |
| Vulva               | -   | 0.46   | 11-16                               | 12.28                               | 0.43-0.50                                 |
| Tail**              | -   | -  | -                                   | 0.52                                | 0.28-0.61                                 |
| Eggs                | 0.045 x 0.026                               | 0.05 x 0.036                                 | 0.040-0.052 x 0.023-0.030           | 0.039 x 0.019                       | 0.043-0.50x                               |
| Uteri branch        | 2   | 2  | 2                                   | -                                   | 4-5                                       |
| Host                | <i>Holochilus brasiliensis</i>              |  |                                     |                                     |   |
| Site of infection   | (= <i>Mus brasiliensis</i> )<br>Stomach     | <i>Nectomys squamipes</i><br>Stomach         | <i>Sigmodon hispidus</i><br>Stomach | <i>Calomys laucha</i><br>Stomach    | <i>Cerradomys subflavus</i><br>Stomach    |
| Locality            | Brazil                                      | Brazil                                       | USA                                 | Argentina                           | Brazil                                    |

All measurements are in millimetres. \*Distance from the anterior end; \*\*Distance from the anus to the posterior end

*Physaloptera turgida* Rudolphi, 1819 to the new genus.

Yamaguti (1961) agreed with the systematic classification of *Physaloptera* proposed by Travassos (1920). More recently, Anderson *et al.* (2009) accepted the presence of two to four uterus as generic characteristics of *Physaloptera*, while *Turgida* is characterized by having more than four uterus. However, Ortlepp (1922) stated that *Physaloptera* and *Turgida* share generic traits, with the differences being restricted to polidelfy. Recently, Pereira *et al.* (2012) reorganized the species of *Physaloptera* according to the number of uterus and divided it into 58 didelphy species, two tridelfys and seven tetradelfy. For other species, the type of uterus is unknown. *P. galvaei* sp. n. presents morphological and morphometric characteristics that allow classifying it within *Physaloptera* genus, particularly by having 4 to 5 uterus (tetra and pentadelfy). The main characteristics of *P. galvaei* sp. n. include spicules sub-equal and not long, three pairs of postcloacal papillae equidistant to each other: first and second pairs asymmetrically displaced (left one anterior to right one), third pair symmetrical with a median protuberance-like papilla in males, and uterus with four or five branches in females.

According to Schell (1950) and Sutton (1989), in comparison with the other species of *Physaloptera* that parasitize rodents sigmodontinae and Eumysopinae in the Americas, the new species can be differentiated from congeners as follows: *P. bispiculata*, *P. murisbrasiliensis*, *P. calnuensis*, *P. longispicula*, and *P. hispida* because it is the only one to have short spicule and to present first and second pairs of papillae asymmetrically displaced (left one anterior to right one) in male. In addition, *P. galvaei* n. sp. resembles *P. hispida* when considered the ratio between body length/size spicule. However *P. hispida* displays twice the size *P. galvaei* body,

and tetra and pentadelfy in female, therefore representing a new species for the genus.

Some authors consider the presence of a papilla-like protuberance in *Physaloptera*, whereas in some original descriptions it is depicted but not considered as a papilla, or probably overlooked. In fact, *P. galvaei* n. sp. share this characteristic with *P. bispiculata*, *P. hispida*, and *P. calnuensis* (Mafra & Lanfredi, 1998; Pereira *et al.*, 2012). There are few studies about SEM comparing *Physaloptera*. SEM studies of *Physaloptera* have reported the presence of small teeth in the external lateral cuticular fold on each side of the pseudolabia. *Physaloptera rara* Hall and Wigdor, 1818 (Naem & Asadi, 2013), *Physaloptera clausa* Rudolphi, 1819 (Gorgani *et al.*, 2013) and *Physaloptera herthameyeriae*. Torres *et al.* (2009) showed only one small tooth on the external lateral cuticular fold on each side of the pseudolabia, although *P. bispiculata* Vaz and Pereira, 1935 (Mafra & Lanfredi, 1998) and *P. galvaei* n. sp. showed a small tooth on the external lateral cuticular fold on each side of the pseudolabia.

Thus, the presence of specific characteristics such as size, shape and ratio of spicule length to total body length, three pairs of postcloacal papillae equidistant to each other: first and second pairs asymmetrically displaced (left one anterior to right one), third pair symmetrical with a median papilla-like protuberance in males, and uterus with four to five branches in females, allow us to consider the specimens from *C. subflavus* as a new species.

Despite the consistency of the specific morphological characters in *Physaloptera* species, will be important for expanding understanding adopt an integrated approach including molecular data.

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Received October 23, 2015.  
Accepted December 4, 2015.