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# A new species of *Neoechinorhynchus* (Eoacanthocephala: Neoechinorhynchidae) from *Pachyurus bonariensis* (Perciformes: Sciaenidae) from the Paraná River basin in Argentina, with comments on two other species of the genus

Nathalia J. ARREDONDO & Alicia A. GIL DE PERTIERRA
Laboratorio de Helmintología, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, Int. Güiraldes 2160, Pabellón II, 4° Piso, Universidad de Buenos Aires, C1428EGA - Buenos Aires, Argentina.
Corresponding author: arredondonj@bg.fcen.uba.ar

**A new species of *Neoechinorhynchus* (Eoacanthocephala: Neoechinorhynchidae) from *Pachyurus bonariensis* (Perciformes: Sciaenidae) from the Paraná River basin in Argentina, with comments on two other species of the genus. -** *Neoechinorhynchus* (*Hebesoma*) *colastinense* sp. n. is described from the intestine of *Pachyurus bonariensis* a freshwater fish from Paraná River basin. The new species is characterized by having a cylindrical trunk, elongated but swollen anteriorly; a spherical proboscis with a prominent apical organ; the anterior circle of hooks very large, alternating in 2 levels, separated from more posterior circles of hooks, but sometimes surpassing the hooks of the middle and posterior circles; a relatively long neck; the male reproductive system occupying 78-81% (79%) of the trunk length; the female reproductive system occupying 27-39% (32%) of the trunk length; and eggs elongated with polar prolongation of the fertilization membrane. Members of the species can be distinguished easily from the other species of South American *Neoechinorhynchus* Stiles & Hassall, 1905 because they are the only ones with characteristics of the subgenus *Hebesoma*. Additionally, two other species are recorded for the first time in Argentina: *Neoechinorhynchus* (*Neoechinorhynchus*) *macronucleatus* Machado Filho, 1954 from *Lycengraulis grossidens* and *N.* (*N.*) *pimelodi* Brasil-Sato & Pavanelli, 1998 from several species of *Pimelodus*. The presence of an apical organ at the proboscis tip is recorded in the new species and *N.* (*N.*) *macronucleatus*. Studies using scanning electron microscopy revealed the presence of non-rimmed pores in the tegument throughout the trunk, neck and proboscis of all three species. A key to the South American species of *Neoechinorhynchus* is provided.

**Keywords:** Taxonomy - morphology - Acanthocephala - *Neoechinorhynchus* - *Hebesoma* - freshwater fishes - South America.

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## INTRODUCTION

Nine out of the more than ninety valid species of *Neoechinorhynchus* Stiles & Hassall, 1905 are known from South America (Amin, 2002; Amin & Heckman, 2009; Salgado-Maldonado *et al.*, 2010). To date, only one species of *Neoechinorhynchus* has been reported from Argentina, named *N.* (*N.*) *villoldoi* Vizcaíno, 1992 from *Corydoras paleatus* (Jenyns, 1842) (see Vizcaíno, 1992; Amin, 2002).

As part of a study on the Acanthocephala in Argentina, the examination of freshwater fishes collected in the Paraná River basin revealed the presence of a previously unknown species of *Neoechinorhynchus* and two known species that are recorded for the first time in Argentina. The objective of this study is to describe the new species and to give new data on other species based on recently collected material. Additionally, scanning electron microscopy micrographs, infection indices and comments on the spectrum of hosts of these species are provided.

## MATERIAL AND METHODS

Fish were caught by local fishermen using hook and line and examined for parasites immediately after capture at the following localities: i) Colastiné River (tributary of the Paraná River) (-31.6696º -60.6078º), Santa Fe Province (Middle Paraná River basin) from 2000 to 2010 [2 *Pachyurus bonariensis* Steindachner, 1879, 80 *Pimelodus albicans* (Valenciennes, 1840), 14 *P. argenteus* Perugia, 1891 and 276 *P. maculatus* Lacépède, 1803]; ii) Paraná-Guazú River (tributary of the Paraná River) (-33.9086° -58.8822º), Entre Ríos Province (Low Paraná River basin) from 2003 to 2010 [26 *Lycengraulis grossidens* (Agassiz, 1829), 15 *P. albicans*, 3 *P. argenteus* and 27 *P. maculatus*].

The acanthocephalans found in the intestine were washed in saline solution, relaxed in cold distilled water for 12 h, fixed in 4% formaldehyde solution and stored in 70% ethanol. Entire worms were hydrated through a gradual ethanol series and cleared and mounted temporarily in Amman's lactophenol, which allows the rotation of the specimen examined. Other specimens were stained with Langeron's alcoholic chlorhydric carmine (Langeron, 1949), differentiated in acid ethanol, dehydrated through a gradual ethanol series, cleared in beechwood creosote and finally mounted in Canada balsam. Eggs laid spontaneously during relaxation of the worms were fixed in 4% formaldehyde solution and examined in distilled water for drawing.

One specimen of the new species from *P. bonariensis*, 1 specimen of *N.* (*N.*) *macronucleatus* from *L. grossidens* and 2 specimens of *N.* (*N.*) *pimelodi* from *P. maculatus* from Argentina preserved in 70% ethanol were prepared for scanning electron microscopy (SEM) as follows: post-fixed in 1% osmium tetroxide, dried with hexamethyldisilazane (Riedel-De Haën®), mounted on stubs with adhesive tape, sputter coated with gold in a Thermo VG Scientific Polaron SC 7630 and examined with a Philips XL 30 scanning electron microscope.

The type material of the new species was deposited in the Parasitological Collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires (MACN-Pa) and in the Natural History Museum, Geneva, Switzerland (MHNG INVE), and voucher material of *N.* (*N.*) *macronucleatus* and *N.* (*N.*) *pimelodi* was deposited in MACN-Pa.

Measurements include the range followed by the mean and number of measurements (n) in parentheses. All measurements are in micrometers (μm) unless otherwise stated. Trunk length excludes neck, proboscis, and bursa. Illustrations were made with the aid of a camera lucida attached to a Zeiss Axioskop microscope equipped with differential interference contrast optics. Abbreviation: LWR, length to width ratio (sensu Monks *et al.*, 2011) and cn, common name. The classification and authorities of the fish follow FishBase (Froese & Pauly, 2012). Prevalence (P), mean intensity of infection (I), and mean abundance (A) were calculated according to Bush *et al.* (1997).

## RESULTS

A new species of *Neoechinorhynchus* allocated to the subgenus *Hebesoma* is described herein. In addition, *Neoechinorhynchus* (*Neoechinorhynchus*) *macronucleatus* and *N.* (*N.*) *pimelodi* are recorded for the first time in the Paraná River basin. Comparative data from these species is given in Table 1.

### *Neoechinorhynchus* (*Hebesoma*) *colastinense* n. sp. Figs 1-8, 10, 13, 16, 19

TYPE MATERIAL: Holotype MANC-Pa No. 517/1 (male); allotype MANC-Pa No. 517/2 (female) and paratypes, MANC-Pa No. 517/3 (4 females) and MHNG INVE 79181 (1 male and 2 females) from *Pachyurus bonariensis*; Colastiné River, Santa Fe Province, Argentina.

ETYMOLOGY: The species is named after the type locality and means "from Colastiné."

#### DESCRIPTION

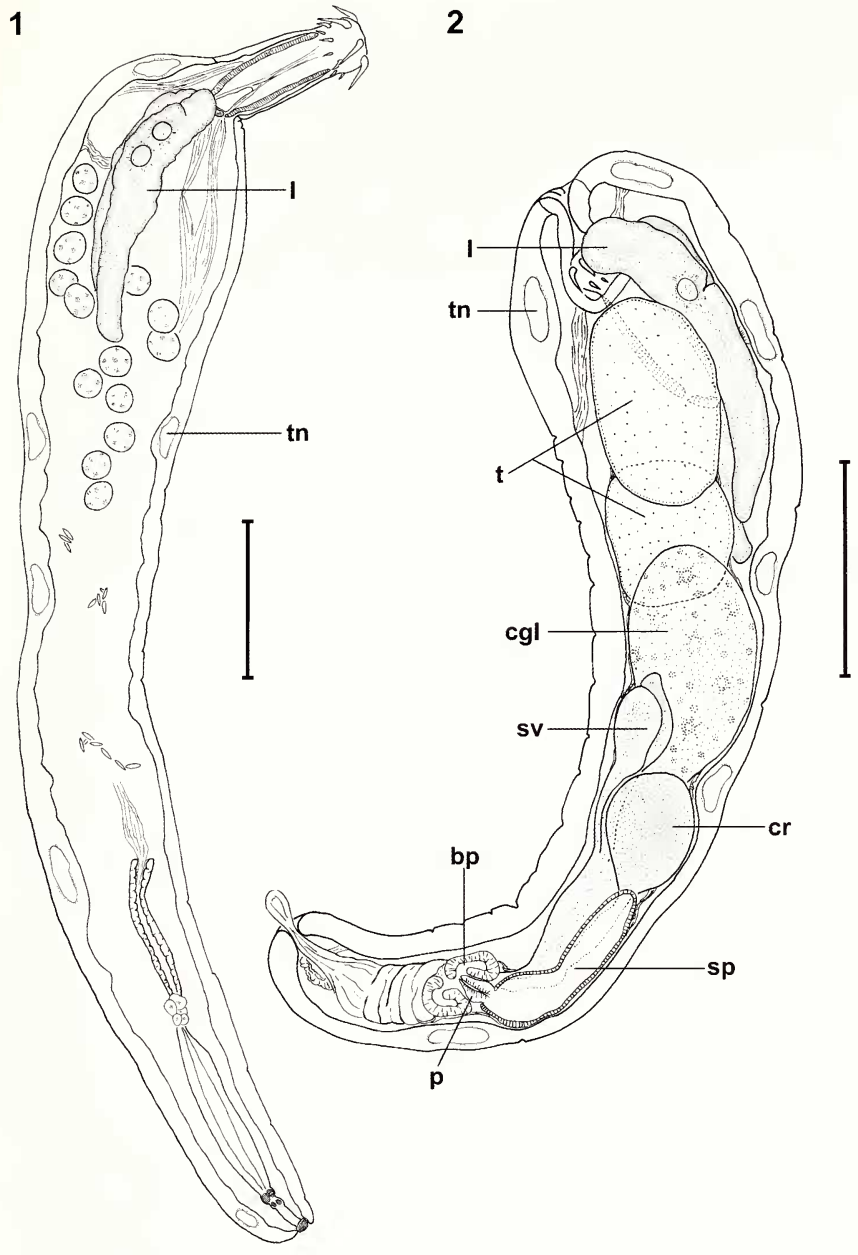
*General* (based on 10 specimens: 2 males, 7 gravid females and 1 juvenile female with ovarian balls used for SEM): Eoacanthocephala, Neoechinorhynchidae, with the characters of the genus *Neoechinorhynchus* and subgenus *Hebesoma* Van Cleave, 1928 (sensu Salgado-Maldonado, 1978; Amin, 2002). Fresh individuals white. Worms small. Trunk cylindrical, elongated, swollen anteriorly, curved ventrally, with 5 dorsal and one ventral giant nuclei, dorsal and ventral body wall similar in thickness (Figs 1, 2). Entire epidermal surface porous (Fig. 19). Sexual dimorphism usually inconspicuous. Proboscis spherical, wider than long (Fig. 3), with prominent apical organ (Fig. 10). Proboscis hooks in 3 circles of 6 hooks each. Hooks in anterior circle largest, alternating in two levels, separated from more posterior circles of hooks but sometimes surpassing the hooks of the middle and posterior circles, with simple roots directed posteriorly. Hooks of the middle and posterior circles much smaller than those of the anterior circle, with orbicular roots (Figs 3, 13). Neck relatively long, broader at base (Figs 1, 10, 16). Proboscis receptacle long, single-walled, extending for a short distance into the trunk when specimens are relaxed (Figs 1, 10); cerebral ganglion pyramidal-shaped situated near posterior end of receptacle (Figs 4, 10). Lemnisci subequal, digitiform, longer than proboscis receptacle, double-nucleated lemnisci usually slightly longer than single-nucleated lemnisci (Figs 1, 2, 4). Genital pore terminal in males and slightly subterminal in females (Figs 1, 2, 5).

*Male*: Trunk 2.6-3.2 mm (n = 2) long, 0.6-0.7 mm wide, LWR 5:1 (Fig. 2). Proboscis 90-110 (n = 2) long, 120-130 wide. Length of proboscis hooks in anterior

TABLE 1. Comparative records of hosts, localities and infection indices for three species of *Neoechinorhynchus* spp. found in Argentina and Brazil; data from Machado Filho (1954), Fabio (1983), Brasil-Sato & Pavanelli (1998, 1999), Santos & Brasil-Sato (2004) and present study.

| Parasite species | Host species | Locality | Site of infection | P (%) | I | A |
|---|---|---|---|---|---|---|
| *Neoechinorhynchus* (*Hebesoma*) *colastinense* n. sp. | *Pachyurus bonariensis** (Perciformes: Sciaenidae) (cn: corvina de río) | Colastiné River, Santa Fe Province, Argentina** | middle intestine | 50 (1/2) | 10 | 0.5 |
| *Neoechinorhynchus* (*Neoechinorhynchus*) *macronucleatus* | *Lycengraulis* sp.* (Clupeiformes: Engraulidae) | Juparaña lagoon, Espírito Santo State, Brazil** | intestine | -- | -- | -- |
| | *Hoplias malabaricus* (Characiformes: Erythrinidae) (cn: tararira) | Municipio de Campos, Rio de Janeiro State, Brazil | intestine | 2.2 (1/45) | 1 | 0.02 |
| | *Lycengraulis grossidens* (Clupeiformes: Engraulidae) (cn: anchoíta) | Paraná-Guazú River, Entre Ríos Province, Argentina | intestine | 7.7 (2/26) | 2.5 (2-3) | 0.2 |
| *Neoechinorhynchys* (*Neoechinorhynchus*) *pimelodi* | *Pimelodus maculatus** (Siluriformes: Pimelodidae) (cn: bagre amarillo) | São Francisco River basin, Três Marias, Minas Gerais, Brazil** | anterior intestine | 39.3 (94/239) | 4.6 | 1.8 |
| | *Franciscodoras marmoratus* (Siluriformes: Doradidae) (cn: bacu pedra) | Três Marias Dam, Minas Gerais State, Brazil | intestine | 10 | 4.3 (1-15) | 0.4 |
| | *Pimelodus albicans* (Siluriformes: Pimelodidae) (cn: bagre blanco) | Colastiné River, Santa Fe Province, Argentina | anterior intestine | 2.5 (2/80) | 6.5 (2-11) | 0.2 |
| | *Pimelodus argenteus* (Siluriformes: Pimelodidae) (cn: bagre) | Colastiné River, Santa Fe Province, Argentina | anterior intestine | 7.1 (1/14) | 2 | 0.1 |
| | *Pimelodus maculatus* | Colastiné River, Santa Fe Province, Argentina | anterior intestine | 2.9 (8/276) | 4.4 (1-19) | 0.1 |

*Type host **Type locality




FIGS 1-2

*Neoechinorhynchus* (*Hebesoma*) *colastinense* n. sp. from *Pachyurus bonariensis*. (1) Entire female worm, lateral view (allotype MACN-Pa 517/2). (2) Entire male worm with withdrawn proboscis, lateral view (holotype MACN-Pa 517/1). *Abbreviations*: bp = bursal pocket; cgl = cement gland; cr = cement reservoir; l = lemniscus; p = pennis; sp = Saeftigen pouch; sv = seminal vesicle; t = testes; tn = tegumental nuclei. Scale-bars 1-2 = 500 μm.

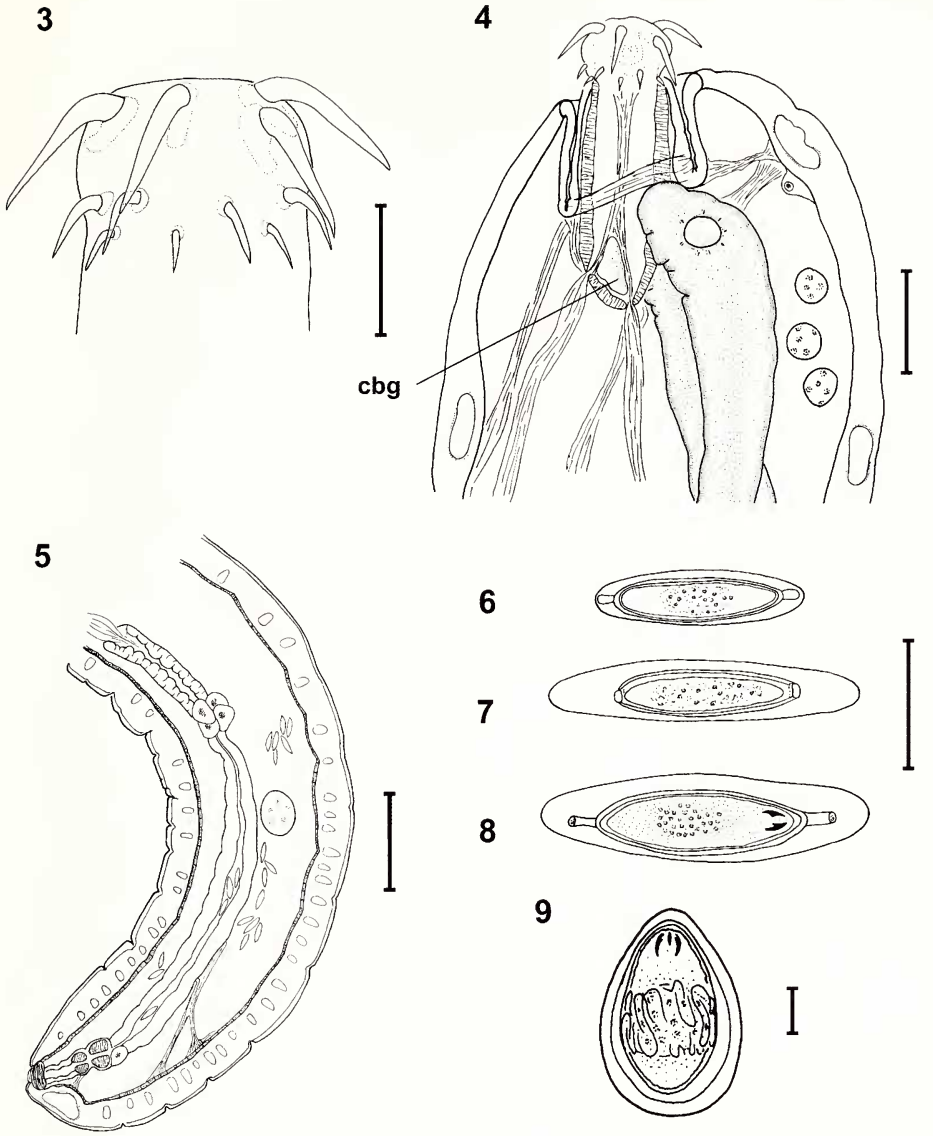
circle 80-95 (85; n = 4), in middle circle 40-45 (n = 2), in posterior circle 15-20 (n = 2); roots not measured. Apical organ 100 long (n = 1), 60 wide. Neck 285-300 (n = 2) long. Proboscis receptacle 350-360 (n = 2) long, 145-150 wide; cerebral ganglion 100-105 (n = 2) long, 35-70 wide. Lemnisci 815-1110 (975; n = 4) long, 150-200 (165) wide. Reproductive system approximately fills the trunk, testes overlap lemnisci, 2.1-2.5 mm (n = 2) in length, occupying 78-81% (79%) of total length. Testes oval, in tandem, overlapping, about equal in size but anterior testis slightly larger, 450-560 (n = 2) long, 300 wide, than posterior 365-530 (n = 2) long, 290-320 wide. Cement gland ovoid, about same size as testes, overlapping posterior testes, 480-535 (n = 2) long, 315-340 wide, with ovoid cement reservoir 280-300 (n = 2) long, 200-245 wide. Saefftigen's pouch 450-485 (n = 2) long, 120-165 wide. Penis 60-90 (n = 2) long, 30-50 wide. Bursa 420-460 (n = 2) long, 210 wide, with two bursal pockets (Fig. 2).

*Female*: Trunk 3.3-4.3 mm (3.9; n = 7) long, 0.6-0.8 mm (0.7) wide, LWR 5-6:1 (Fig. 1). Proboscis 115-140 (130; n = 4) long, 160-170 (165) wide. Length of proboscis hooks in anterior circle 100-115 (105; n = 7), in middle circle 40-55 (45; n =7), in posterior circle 20-45 (35; n = 7); length of hook roots in anterior circle 50-60 (55; n = 6), in middle circle 10-20 (15; n = 6), in posterior circle 5-15 (10; n = 6). Apical organ 95-120 (105; n = 5) long, 55-85 (75) wide. Neck 36-475 (440; n = 3) long, 205-210 (n = 2) wide. Proboscis receptacle 435-475 (455; n = 7) long, 140-170 (155) wide; cerebral ganglion 95-150 (120; n = 7) long, 45-60 (55) wide. Lemnisci 775-1245 (1065; n = 14) long, 145-190 (175) wide (Figs 1, 4). Reproductive system length 1.15-1.35 mm (1.25; n = 7), occupying 27-39% (32%) of total trunk length. Uterine bell 300-600 (435; n = 6) long, 50-100 (75) wide; uterus elongated 580-700 (660; n = 7) long, 80-115 (100) wide; vagina 160-200 (185; n = 7) long, 55-65 (60) wide (Figs 1, 5). Eggs elongated, outer membrane 40-65 (60; n = 10) long, 10-20 (15) wide; fertilization membrane with polar prolongations 40-55 (50; n = 10) long, 10-15 (11) wide; acanthor 30-40 (35; n = 10) long, 8-13 (9) wide; larval hooks 2-4 (3; n = 13) long (Figs 6-8).

DIFFERENTIAL DIAGNOSIS: The new species is characterized by the following combination of features: a cylindrical trunk, elongated and swollen anteriorly; a spherical proboscis with a prominent apical organ; an anterior circle of hooks very large, sometimes overlapping the middle and posterior circles of hooks; a relatively long neck; a male reproductive system occupying 78-81% (79%) of the total trunk length; a female reproductive system occupying 27%-39% (32%) of the trunk length; and elongated eggs with polar prolongations of fertilization membrane.

The eggs dispersed in the trunk cavity show different developmental stages of the polar prolongations of the fertilization membrane, from eggs without prolongations to eggs with prolongations not fully developed (Figs 6, 7). All the eggs measured in this description, which were those ripe and spontaneously laid during the fixation of adults, had such prolongations (Fig. 8).

*Neoechinorhynchus* (*H.*) *colastinense* sp. n. differs from all the South American neoechinorhynchids because it belongs to the subgenus *Hebesoma* (egg with polar prolongations of the fertilization membrane) (sensu Salgado-Maldonado, 1978; Amin, 2002), and by the large percentage of trunk cavity occupied by the female reproductive system (32%).




FIGS 3-9

(3-8) *Neoechinorhynchus* (*Hebesoma*) *colastinense* sp. n. from *Pachyurus bonariensis*. (3) Armature of female proboscis (allotype MACN-Pa 517/2). (4) Detail of anterior region of trunk of the female worm, lateral view showing proboscis partially withdrawn and proboscis receptacle (paratype MACN-Pa 517/3). (5) Detail of female reproductive system, lateral view (paratype MACN-Pa 517/3). (6-8) Detail of eggs in different developmental stages of the polar prolongations of the fertilization membrane. (9) *Neoechinorhynchus* (*Neoechinorhynchus*) *pimelodi* from *Pimelodus maculatus*. Detail of drop-shaped egg. *Abbreviations*: cbg = cerebral ganglion. Scale-bars 3 = 100 μm; 4-5 = 200 μm; 6-8 = 25 μm; 9 = 5 μm.

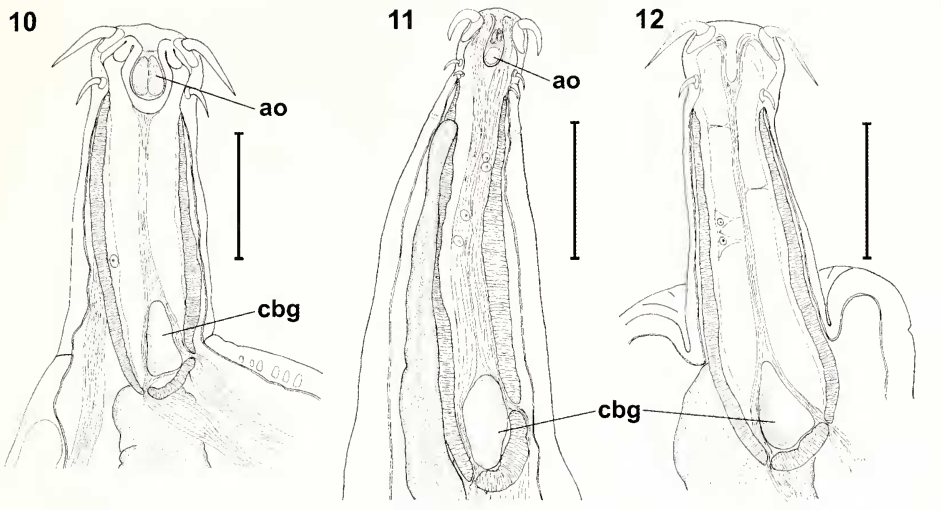
Only twelve of the more than ninety species of *Neoechinorhynchus* were placed on the subgenus *Hebesoma*, including species parasites of fishes and turtles from North America, Asia and India (Amin, 2002; Amin & Muzzall, 2009). Using Amin´s key (see Amin, 2002), it is possible to discriminate the new species from the following North American species belonging to *Hebesoma* from fishes: *N.* (*H.*) *agilis* (Rudolphi, 1819) (with holarctic distribution), *N.* (*H.*) *carinatus* Buckner & Buckner, 1993, *N.* (*H.*) *didelphis* Amin, 2001, *N.* (*H.*) *doryphorus* Van Cleave & Bangham, 1949, *N.* (*H.*) *idahoensis* Amin & Heckmann, 1992, *N.* (*H.*) *pungitius* Dechtiar, 1971, and *N.* (*H.*) *rostratus* Amin & Bullock, 1998. *Neoechinorhynchus* (*H.*) *colastinense* sp. n. differs from *N.* (*H.*) *agilis* by having a shorter trunk length (2.6-4.3 versus up to 11.2) and the number of giant hypodermal nuclei (5 dorsal and one ventral versus 6 dorsal and two ventral); from *N.* (*H.*) *carinatus* and *N.* (*H.*) *doryphorus* by the length of the anterior circle of hooks (same length versus lateral anterior hooks longer than other hooks in the same circle); from *N.* (*H.*) *didelphis* by having a single uterine bell and the lack of neck girdle; from *N.* (*H.*) *idahoensis* by the length of hooks in anterior and middle circles (anterior circle of hooks much larger than the middle circle versus anterior and middle circles of similar length); from *N.* (*H.*) *pungitius* by having a larger proboscis in males and females (90-110 long, 120-130 wide and 115-140 long, 160-170 wide versus 57-90 long, 79-95 wide and 63-90 long, 84-118 wide), and the polar prolongations of fertilization membrane not extending to the outer shell; and from *N.* (*H.*) *rostratus* by having hooks rooted in all circles (versus only the anterior circle rooted). The new species differs from *N.* (*H.*) *tenellus* (Van Cleave, 1913), recently placed in *Hebesoma* (sensu Amin & Muzzall, 2009), mainly by having a longer neck (versus short), and a greater percentage of the trunk cavity occupied by the female reproductive system (32% versus 12%, respectively).

***Neoechinorhynchus* (*Neoechinorhynchus*) *macronucleatus* Machado Filho, 1954**
Figs 11, 14, 17

MATERIAL STUDIED: MANC-Pa No. 518/1-2 (2 females) from *Lycengraulis grossidens*; Paraná-Guazú River, Entre Ríos Province, Argentina.

REMARKS: Machado Filho (1954) briefly described this species from the intestine of *Licengraulis* sp. from Brazil. Later, Fabio (1983) recorded one male specimen from *Hoplias malabaricus* (Bloch, 1794) also from Brazil. In Argentina, only five juvenile females were recovered from *L. grossidens*. The specimens are easily recognized because they have a cylindrical proboscis, the hooks of the anterior circle larger and stouter than the hooks in the middle and posterior circles, four prominent giant nuclei pre-equatorially situated in the dorsal body wall and lemnisci much longer than the proboscis receptacle.

In addition, minor differences were recorded in the measures of some characters while others were recorded for the first time: proboscis 110-135 (n = 2) long, 95-110 wide; length of hooks in anterior circle 40-50 (45; n = 4), in middle circle 25-35 (30; n = 4), in posterior circle 15-25 (20; n = 4); length of hook roots in anterior circle 40-50 (45; n = 4), in middle circle 5-15 (10; n = 3), in posterior circle 5 (n = 3); apical organ 65-75 (n = 2) long, 25 wide; proboscis receptacle 435-560 (n = 2) long, 110-125 wide; cerebral ganglion oval-shaped, situated near posterior end of receptacle 105-130




FIGS 10-12

*Neoechinorhynchus* spp. females, details of anterior region of trunk. (10) *Neoechinorhynchus* (*Hebesoma*) *colastinense* sp. n. from *Pachyurus bonariensis* (allotype MACN-Pa 517/2). (11) *N.* (*Neoechinorhynchus*) *macronucleatus* from *Lycengraulis grossidens* (voucher MACN-Pa 518/1-2). (12) *N.* (*N.*) *pimelodi* from *Pimelodus maculatus* (voucher MACN-Pa 519/3). *Abbreviations*: ao = apical organ; cbg = cerebral ganglion. Scale-bars 10-12 = 200 μm.

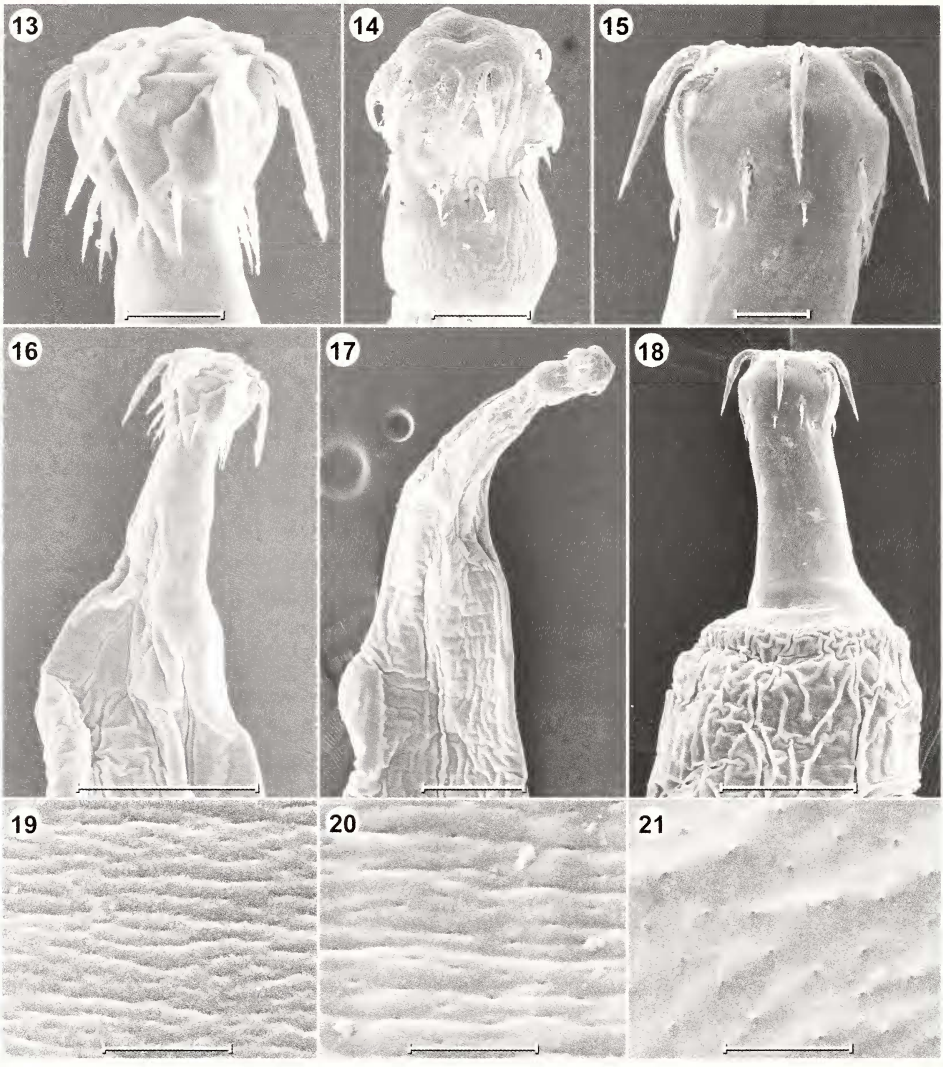
(n = 2) long, 50-65 wide (Figs 11, 14, 17). The presence of an apical organ, cerebral ganglion and roots of hooks in middle and posterior circle are recorded for the first time, and this is the first study of *N.* (*N.*) *macronucleatus* using SEM.

***Neoechinorhynchus* (*Neoechinorhynchus*) *pimelodi* Brasil-Sato & Pavanelli, 1998**
Figs 9, 12, 15, 18, 20, 21

MATERIAL STUDIED: MANC-Pa No. 519/1 (3 males and 5 females) from *P. albicans;* MANC-Pa No. 519/2 (2 females) from *P. argenteus*; and MANC-Pa No. 519/3 (6 males and 7 females) from *P. maculatus*; Colastiné River, Sante Fe Province, Argentina.

REMARKS: This species was originally described by Brasil-Sato & Pavanelli (1998) from *Pimelodus maculatus* and later from *Franciscodoras marmoratus* (Lütken, 1874) (Siluriformes: Doradidae) by Santos & Brasil-Sato (2004), both from São Francisco River in Brazil. In Argentina, this species was found in the type host *P. maculatus* and also in two previously unrecorded hosts, *P. albicans* and *P. argenteus*; all of the hosts belong to the Pimelodidae. This is also the first record of this species from the Paraná River basin. The SEM studies on this species made by Brasil-Sato & Pavanelli (1998) showed only the copulatory bursa, but herein we include the proboscis, anterior trunk extremity, and the porous tegumental surface (Figs 15, 18, 20, 21).

The specimens from Argentina are larger than those from Brazil. Some of the differences recorded in males and females are, for example, length of trunk (2.8-6.3 mm and 2.2-6.8 mm, respectively), proboscis dimensions (115-175 long, 120-195 wide and 135-175 long, 130-200 wide, respectively), proboscis receptacle length (450-635




FIGS 13-21

*Neoechinorhynchus* spp. females, SEM micrographs. (13-15) Detail of proboscis. (13) *N.* (*Hebesoma*) *colastinense* sp. n. (14) *N.* (*Neoechinorhynchus*) *macronucleatus*. (15). *N.* (*N.*) *pimelodi*; (16-18). Anterior trunk and neck. (16) *N.* (*H.*) *colastinense*. (17) *N.* (*N.*) *macronucle - atus*. (18) *N.* (*N.*) *pimelodi*. (19-21) Detail of porous tegument. (19) *N.* (*H.*) *colastinense* sp. n., anterior trunk. (20) *N.* (*N.*) *pimelodi*, anterior trunk. (21) *N.* (*N.*) *pimelodi*, posterior trunk. Scale-bars 13-15 = 50 μm; 16-18 = 200 μm; 19-21 = 1 μm.

and 450-630, respectively) (Fig. 12), and lemnisci length (980-2500 and 1020-2220, respectively). Brasil-Sato & Pavanelli (1998) measured only the outer membrane of the eggs, now the size of each component of the eggs was determined: outer membrane 22–25 (24; n = 4) long, 15-17 (16) wide versus 15-22 (18) long, 12-15 (14) wide in the

Brazilian specimens; fertilization membrane 21-23 (22; n = 4) long, 13-15 (14) wide; acanthor 18-21 (19; n = 4) long, 10-12 (11) wide; larval hooks 2-4 (3; n = 5) long (Fig. 9). The eggs of the new material are slightly larger than those from Brazil. This difference could be because the eggs measured by Brasil-Sato & Pavanelli (1998) are not completely mature (intrauterine or free in trunk cavity) versus spontaneously laid eggs in this work. However, the similarities in the shape of the trunk (elliptic), the shape of the proboscis (spherical), the apparent absence of the apical organ, the distribution and size of the proboscis hooks, the percentage of the trunk occupied by the reproductive system and the position of the genital pore in males and females, and particularly in the morphology of the eggs (drop-shaped) allowed assigning the specimens from Argentina to *Neoechinorhynchus* (*N.*) *pimelodi*. Thus, size differences could be due to differential growth rates from different hosts (see Amin & Muzzall, 2009). The low indices of infection (prevalence, mean intensity and mean abundance) recorded in all the hosts collected in the Paraná River basin do not allow establishing which the principal host is. Brasil-Sato & Pavanelli (1999) studied the prevalence and mean intensity of infection of *N.* (*N.*) *pimelodi* from *P. maculatus* in the São Francisco River (Brazil) during the drought and flooding period; its prevalence is much higher than that in Colastiné River (42-51% and 30-34% versus 2.9% in the present paper), whereas the mean intensity of infection is very similar (4.8-4.9 and 3.5-4.9 versus 4.4 in the present paper).

## DISCUSSION

Salgado-Maldonado (1978) proposed the synonymy of the genus *Neoechinorhynchus* Stiles & Hassal, 1905 with *Hebesoma* Van Cleave, 1928. This synonymy was accepted by Amin (2002), who recognized *Hebesoma* as a subgenus of *Neoechinorhynchus* based on the polar prolongations of the egg fertilisation membrane. Subsequently, most authors accepted the use of the subgenus as an additional feature for species characterization and discrimination (e.g. Amin *et al*., 2003; Barger *et al*., 2004; Barger & Nickol, 2004; Barger, 2004; 2005; Amin & Christinson, 2005; Mikhailova & Atrashkevich, 2008; Amin & Muzzall, 2009; Amin & Heckman, 2009; Martínez-Aquino *et al*., 2009; Salgado-Maldonado *et al*., 2010). In agreement with these authors, the new species is here allocated to the subgenus *Hebesoma*.

None of the nine species of *Neoechinorhynchus* described from South America, except the new one described herein, belongs to the subgenus *Hebesoma* (eggs with polar prolongations of the fertilization membrane). Considering the importance of the morphology of the eggs to discriminate between species belonging to the subgenus *Neoechinorhynchus* or *Hebesoma*, it will be interesting to study only completely ripe eggs (see Fig. 8, present paper) of the South American species that were originally described as without polar prolongations.

*Neoechinorhynchus* (*Neoechinorhynchus*) *golvani* was originally described from Mexico. Later, Brasil-Sato & Pavanelli (1998) mentioned the occurrence of this parasite from the Amazon River in Brazil and Amin (2002) also mentioned *N.* (*N.*) *golvani* as present in Mexico and Brazil. Curiously, Thatcher (2006) only mentioned its presence in Mexico, and Portes Santos *et al*. (2008) did not include this species in their exhaustive checklist about the acanthocephalans from Brazil. In view of the fact that

no specimens of this species are deposited in any collection in Brazil (Dr. Knoff, Curator of Instituto Oswaldo Cruz, and Dr. Magalhães Volunteer Curator of Instituto Nacional de Pesquisas da Amazônia, Brazil; pers. comm.) and that almost all records of *N.* (*N.*) *golvani* are from Central America (e.g. Martínez-Aquino *et al.*, 2009; Monks *et al.*, 2011; Salgado-Maldonado *et al.*, 2010), the occurrence of *N.* (*N.*) *golvani* in South America should be considered as an erroneous reference.

The presence of an apical organ at the proboscis tip was infrequently recorded among South American species of *Neoechinorhynchus*, only two of 9 species (*N.* (*N.*) *buttnerae* and *N.* (*N.*) *curemai*). In this work, two of the 3 species studied [*N.* (*H.*) *colastinense* and *N.* (*N.*) *macronucleatus*] have an apical organ.

Among South American species of *Neoechinorhynchus*, only *N.* (*N.*) *curemai* and *N.* (*N.*) *pimelodi* have been studied with SEM (Brasil-Sato & Pavanelli, 1998; Martins *et al.*, 2000). Amin & Heckmann (2009) reported the presence of non-rimmed pores throughout the trunk, neck and proboscis in the tegument of *N.* (*N.*) *buckneri* from the USA; a similar porous surface was observed in the three species studied herein.

During this study, *Neoechynorhynchus* (*H.*) *colastinense* and *N.* (*N.*) *macronucleatus* were found only parasitizing *P. bonariensis* and *L. grossidens*, respectively, whereas *N.* (*N.*) *pimelodi* was found widespread in *P. albicans*, *P. argenteus* and *P. maculatus* from Colastiné River. However, *Pomphorhynchus sphaericus* Gil de Pertierra, Spatz & Doma, 1996, a parasite of *P. albicans* and *P. maculatus* from La Plata River in Argentina (Gil de Pertierra *et al.*, 1996), has never been found in Colastiné River.

Key to the South American species of *Neoechinorhynchus*:

1a. Eggs with concentric membranes, without polar prolongations of the fertilization membrane; subgenus *Neoechinorhynchus* Stiles & Hassall, 1905 . . . . . 2

1b. Eggs with polar prolongations of the fertilization membrane; subgenus *Hebesoma* Van Cleave, 1928 . . . . . 9

2a. Trunk ovoid, elliptical or fusiform, swollen equatorially; proboscis without apical organ . . . . . 3

2b. Trunk elongated, swollen anteriorly; proboscis with or without apical organ . . . . . 6

3a. Lemnisci much longer than proboscis receptacle (twice or more); male reproductive system occupies about 50% of trunk . . . . . 4

3b. Lemnisci longer than proboscis receptacle; male reproductive system occupies more than 60% of trunk . . . . . 5

4a. Sexual dimorphisms present; neck twice longer than the proboscis; lemnisci subequal, overlapping anterior testes; eggs drop-shaped . . . . . *N.* (*N.*) *pimelodi* Brasil-Sato & Pavanelli, 1998

4b. Sexual dimorphisms absent; neck short; lenmisci unequal, reaching level of testes, but not overlapping them; eggs elongate . . . . . *N.* (*N.*) *prochilodorum* Nickol & Thatcher, 1971

5a. Neck short; lemnisci slightly longer than proboscis receptacle; cement gland almost same size than testes . *N.* (*N.*) *paraguayensis* Machado Filho, 1959

5b. Neck long; lemnisci much longer than proboscis receptacle; cement gland bigger than testes . . . . . *N.* (*N.*) *pterodoridis* Thatcher, 1981

6a. Proboscis with apical organ not observed; tegument with 1–2 dorsally and 1–3 ventral giant nuclei . . . . . *N.* (*N.*) *villoldoi* Vizcaíno, 1992

6b. Probosis with apical organ; tegument with 5 dorsally and 1–2 ventral giant nuclei . . . . . 7

7a. Dorsal tegument with 4 prominent pre-equatorial giant nuclei and the fifth post-equatorial . . . . . *N.* (*N.*) *macronucleatus* Machado Filho, 1954

7b. Dorsal tegument with giant nuclei not clustered in pre-equatorial region . . . . 8

8a. Apical organ about half length than proboscis; elongated cement gland, separated for a distance of testes. Coiled vagina associated to paravaginal muscles . . . . . *N.* (*N.*) *buttnerae* Golvan, 1956

8b. Apical organ large, almost same length than proboscis; elongated cement gland overlaps testes. Proboscis with two lateral hooks larger than other in first circle . . . . . *N.* (*N.*) *curemai* Noronha, 1973

9a. Proboscis with prominent apical organ; neck relatively long, trunk elongated, swollen anteriorly; male reproductive system 79%, female reproductive system 32% . . . . . *N.* (*H.*) *colastinense* sp. n.

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