

ANA NUNES DOS SANTOS

**NOVA ESPÉCIE DE *Neocosmocercella* BAKER & VAUCHER, 1983
PARASITO DE INTESTINO GROSSO DE *Phyllomedusa vaillantii*
(ANURA: PHYLLOMEDUSIDAE) DA FLORESTA NACIONAL DE
CAXIUANÃ, AMAZÔNIA ORIENTAL, BRASIL**

BELÉM

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Trabalho de Conclusão de Curso apresentado ao Colegiado do Curso de Bacharelado em Ciências Biológicas, modalidade Biologia da Universidade Federal do Pará, como requisito parcial para obtenção do grau de Bacharel em Biologia.

Orientador: Prof. Dr. Francisco Tiago de Vasconcelos Melo, Instituto de Ciências Biológicas- UFPA

Co-Orientadora: Profa. Cynthia Elizabeth González, Centro de Ecología Aplicada del Litoral (CECOAL), Corrientes, Argentina

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Avaliador: Prof.^a Dra. Roberta Melo Valente

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“A única verdadeira tristeza está na ausência de desejo”

Charles Darwin

Dedico a minha família, amigos, professores e a todos que
Contribuíram com essa conquista.

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RESUMO

Neocosmocercella bakeri sp. n. é um parasito de intestino grosso de *Phyllomedusa vaillantii* Boulenger 1882, da Floresta Nacional de Caxiuanã, Amazônia Oriental Brasileira. O estudo descreve a segunda espécie do gênero que é facilmente diferenciada da espécie tipo, *Neocosmocercella paraguayensis* Baker e Vaucher, 1983, por apresentar uma abertura bucal triangular, com três lábios simples, diferente dos três lábios bilobados e abertura bucal hexagonal de *N. paraguayensis*. Além disso, a nova espécie apresenta papilas somáticas que são ausentes em *N. paraguayensis*. Os machos são diferentes na distribuição das papilas cloacais sésseis e nas dimensões do gubernáculo, assim como as fêmeas diferenciam-se por serem menores e apresentarem úteros opistodelfos. Além da descrição da segunda espécie do gênero, adicionamos dados sobre os caracteres taxonômicos do diagnóstico de *Neocosmocercella*. Este é o primeiro estudo a relatar a infecção de anuros com *Neocosmocercella* na Amazônia brasileira, e o registro de um novo hospedeiro.

Palavras-chave: Nematódeos, Cosmocercoidea, *Neocosmocercella*, Amazônia Oriental

ABSTRACT

Neocosmocercella bakeri sp. n. is a parasite of the large intestine of *Phyllomedusa vaillantii* Boulenger found in the Caxiuanã National Forest in the Eastern Brazilian Amazon. The study describes the second species of the genus is easily distinguished from the type species, *Neocosmocercella paraguayensis* Baker & Vaucher, 1983, by presenting a triangular mouth opening with three simple lips, in contrast to the three bilobed lips and hexagonal mouth opening in *N. paraguayensis*. In addition, the new species has somatic papillae, which are absent in *N. paraguayensis*. The males are distinguished by the distribution of sessile cloacal papillae and the dimensions of the gubernaculum, whereas the females are distinguished by their smaller size and opisthodelphic uterus. Beyond the second species of the genus, we are discussing about taxonomic characters on diagnosis of *Neocosmocercella*. This study is the first to report the infection of anurans with *Neocosmocercella* in the Brazilian Amazon, the record of a new host for the genus.

Keywords: Nematodes, Cosmocercoida, *Neocosmocercella*, Eastern Amazon

APRESENTAÇÃO DO TRABALHO DE CONCLUSÃO DE CURSO

ARTIGO

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Nova espécie de *Neocosmocercella* Baker & Vaucher, 1983 parasito de intestino grosso de *Phyllomedusa vaillantii* (Anura: Phyllomedusidae) da Floresta Nacional de Caxiuanã, Amazônia Oriental, Brasil

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INTRODUÇÃO

Phyllomedusa Wagler, é um gênero de anuros da família Phyllomedusidae, com 16 espécies, ocorrendo desde a América Central até a América do Sul a leste dos Andes, incluindo Trinidad, até o Norte da Argentina e Uruguai (Frost, 2016). *Phyllomedusa vaillantii* Boulenger, é uma espécie de hábito noturno, sendo encontrada geralmente nas folhagens de árvores e arbustos próximos de córregos ou em corpos de água permanentes em florestas tropicais (Duellman, 1978). Sua distribuição na América do Sul ocorre desde as Guianas e Bacia Amazônica da Guiana, Suriname, Guiana Francesa, nordeste do Brasil até a Colômbia, Peru e Norte da Bolívia, Amazonas até a Venezuela (Frost, 2016).

Até o momento, quatro espécies de gêneros de nematódeos foram reportados parasitando *Phyllomedusa* spp.: *Cosmocercella* Steiner, 1924; *Physalopteroides* Wu & Liu, 1940; *Oswaldocruzia* Travassos, 1917; and *Cosmocerca* Diesing, 1861 (Campião et al. 2014). Os gêneros de nematódeos *Cosmocercella* e *Cosmocerca* pertencem a Família Cosmocercidae e são comumente encontrados parasitando intestino de anfíbios (Vicente, et al., 1990). Entre os

gêneros da Família Cosmocercidae, *Neocosmocercella* Baker & Vaucher, 1983, se destaca por ser um gênero monotípico, e a espécie tipo, *Neocosmocercella paraguayensis* Baker & Vaucher 1983, foi encontrada parasitando *Pithecopus hypochondryalis* (Daudin) no Paraguai (Baker e Vaucher 1983).

Apenas espécies de três gêneros de nematódeos, foram registrados como parasitos de *Phyllomedusa vaillantii*: *Cosmocercella phyllomedusae*, *Physalopteroides venancioi* e *Oswaldocruzia* sp. (Campião et al., 2014). O presente estudo descreve uma nova espécie de *Neocosmocercella* e relata o parasitismo desta espécie em *P. vaillantii* na Amazônia Oriental Brasileira.

MATERIAL E MÉTODOS

Dez espécimes de *Phyllomedusa vaillantii* foram coletados na Estação Científica Ferreira Pena (espécimes hospedeiros foram coletados sob licença 0004/06 NUC SUPES PA e SISBIO 32660-1), localizada na Floresta Nacional de Caxiuanã, no Município de Melgaço, Estado do Pará, Brasil, durante uma expedição ocorrida no mês de março de 2015 para a coleta de helmintos parasitos de anfíbios e reptéis. Os hospedeiros foram eutanasiados por injeção de cloridrato de lidocaína a 2%, e os órgãos internos foram dissecados e examinados. Os helmintos foram coletados, colocados em solução salina e fixados em etanol 70% aquecido, e para análises morfológicas e morfométricas, os nematódeos foram clarificados em lactofenol de Amman e analisados através do microscópio Olympus BX41 (Olympus, Tokyo, Japão) acoplado com câmara clara. As estruturas taxonômicas estão em micrômetros, ou indicadas quando de outra forma. Todas as medidas são apresentadas em intervalos, seguidas pela média entre parênteses.

Para a Microscopia Eletrônica de Varredura, as amostras foram pós-fixadas em O₂OS₄ 1%, submetidas a desidratação em série etanólica crescente, até a secagem ao ponto crítico do CO₂, metalizados em ouro, e examinadas em microscópio Vega3 (TESCAN), do Laboratório de Embriologia e Histologia (Laboratório de Embriologia e Histologia / Universidade Federal Rural da Amazônia-UFRA).

Holótipo, Alótipo e Parátipos foram depositados na Coleção de Invertebrados do Museu Emílio Goeldi (Museu Paraense Emílio Goeldi, MPEG) e os hospedeiros foram depositados na coleção herpetológica do MPEG.

RESULTADOS

Ordem Ascaridida Skrjabin and Shulz, 1940

Superfamília Cosmocercoidea Travassos, 1925

Família Cosmocercoidea Travassos, 1925

***Neocosmocercoella* Baker and Vaucher, 1983**

***Neocosmocercoella bakeri* sp. n.**

Hospedeiro: *Phyllomedusa vaillantii* Boulenger, 1882

Sítio de infecção: Intestino grosso

Localidade tipo: Floresta Nacional de Caxiuanã (1°47'32.3"S, 51°26'02.5"W), Pará, Brasil

Prevalência: 30% (3 de 10)

Intensidade média: 409 por hospedeiro infectado (amplitude 5 – 1227)

Abundância média: 129,3 por hospedeiro

Material tipo: Holótipo (MPEG 0068); Alótipo (MPEG 0069); Parátipos (MPEG 0070)

Etimologia: A nova espécie é em homenagem a Michael R. Baker, em reconhecimento de suas muitas contribuições sobre a sistemática e biologia dos parasitas de anfíbios e répteis

Descrição (Figs. 1, 2)

Descrição geral: Nematódeos pequenos e delgados. Dimorfismo sexual evidente, as fêmeas maiores que os machos, machos com a região caudal curvada (Fig.1a, d). Cutícula com delicadas estriações transversais; aleta lateral presente em ambos os sexos, aletas laterais presentes que se iniciam na região do bulbo esofágico e terminam a nível da cloaca nos machos e após o ânus nas fêmeas. Abertura bucal triangular, com três lábios; lábio dorsal com duas papilas cefálicas, cada lábio ventrolateral com uma papila e um par de anfidio (Fig. 1c, 2a). Esôfago composto por faringe, corpus, istmo e bulbo com válvulas quitinosas (Fig. 1b). anel nervoso situado na região mediana do esôfago, poro excretor próximo ao bulbo esofágico (Fig. 1b). Fêmeas didelfas, opistodelfas. Papilas somáticas presentes (Fig. 2b, g, h). Cauda afilada e pontiaguda em ambos os sexos (Fig. 1g, 2g, h).

Machos (baseado em 1 Holótipo e 9 Parátipos). Comprimento total 1,4 – 2,06 (1,6) mm, esôfago 302,5 – 354,5 (341) comprimento incluindo o bulbo, faringe 23 – 30 (27), corpus

204 – 253 (228), istmo 26 – 43 (33), bulbo 47 – 52 (50) comprimento e 38 – 48 (44) largura. Anel nervoso 127 – 148 (139) e poro excretor 200 – 221 (207) da extremidade anterior. Largura do corpo na junção esôfago-intestino 45 – 66 (50). Extremidade posterior curvada ventralmente. Região ventral pré-cloacal com duas fileiras de 25 a 29 papilas vesiculadas (Fig. 2e) que diminuem gradualmente, variando entre 11 a 14 no lado esquerdo e 12 a 15 do lado direito (Fig. 1d, g, 2d). Um par de papilas sésseis pré-cloacais. Lábio anterior da cloaca com dois pares de papilas e uma papila volumosa não pareada; dois pares de papilas ad-cloacais; dois pares de papilas pós-cloacal e um par lateral (Fig. 2f). Espículos subiguais 104 – 139 (113) comprimento (Fig. 1e), gubernáculo côncavo triangular 25 – 32 (31). Cauda afilada, pontiaguda, 126 – 148 (137) comprimento.

Fêmeas (baseado em 1 Alótipo e 9 Parátipos). Corpo inicia afilado e dilata posteriormente, 2,4 – 2,6 (2,5) mm comprimento, largura do corpo na região da vulva 128 – 184 (154). Esôfago 403 – 451 (423) de comprimento incluindo o bulbo; faringe 29 – 37 (33), corpus 267 – 304 (285), istmo 35 – 45 (39), bulbo 59 – 77 (65) de comprimento e 56 – 67 (60) de largura. Anel nervoso 139 – 186 (166) e poro excretor 210 – 288 (265) da extremidade anterior. Largura do corpo na junção esôfago-intestino 91 – 115 (99). Vulva discreta, pre-equatorial (Fig. 1f, 2c), situada a 1.02 – 1.14 (1,08) mm da extremidade anterior. Vagina bem desenvolvida, direcionada anteriormente (*vagina vera*) e flexionada para região posterior (*vagina uterina*) terminando no início do saco uterino muscular (Fig. 1f), ovovivíparas (Fig. 1a). Útero preenchido com 8 a 10 ovos, sendo os últimos ovos com larva (Figs. 1a, h), ovos em mórula 152 – 185 (168) comprimento e 96 – 135 (114) largura. Cauda afilada 224 – 277 (241) de comprimento, pontiaguda.

DISCUSSÃO

A distribuição e forma das papilas ornamentadas são os principais caracteres utilizados para diferenciação de gêneros na subfamília Cosmocercinae (Anderson et al., 2009). Assim, os nematódeos encontrados parasitando o intestino grosso de *Phyllomedusa vaillantii*, no presente estudo, podem ser classificados no gênero *Neocosmocercella* por seus exemplares machos apresentarem somente papilas vesiculadas na região posterior. O gênero *Cosmocercella* Steiner, 1924, se aproxima morfologicamente de *Neocosmocercella* por também apresentar este tipo de papilas, porém são facilmente diferenciados por possuírem ainda papilas em roseta na região posterior dos machos (Baker and Adamson, 1977). Adicionalmente, *Neocosmocercella bakeri*

sp. n., difere das espécies de *Cosmocercella* e das pertencentes aos demais gêneros da subfamília Cosmocercinae por apresentar, vagina uterina alongada e saco uterino nas fêmeas (ausente em outros gêneros).

O gênero *Neocosmocercella* foi proposto por Baker and Vaucher (1983) para acomodar exemplares encontrados no intestino grosso de *Pithecopus hypochondrialis* (Daudin, 1890), no Paraguai. Baker e Vaucher (1983), baseiam-se principalmente em características como a presença de saco uterino, e vagina alongada, machos com região posterior enrolada e papilas vesiculadas na extremidade caudal. Além disso, aspectos relacionados a abertura bucal e posição do útero foram apontados pelo autor como importantes caracteres genéricos: abertura bucal hexagonal, formada por três lábios bilobados e útero prodelfo; todos esses caracteres foram corroborados por Draghi et al. (2015) em espécimes de *N. paraguayensis* encontrados em *Pithecopus azureus* (Cope, 1862) em Formosa, Argentina. Porém, dentre esses caracteres genéricos, *Neocosmocercella bakeri* sp. n. apresenta papilas vesiculadas pré-cloacais nos machos, vagina alongada e saco uterino nas fêmeas; a espécie descrita no presente trabalho possui abertura bucal triangular, lábios simples e fêmeas com úteros opistodelfos. Dessa forma, as características presentes na nova espécie que difere de *N. paraguayensis* amplia os caracteres do gênero.

Neocosmocercella bakeri sp. n. é facilmente diferenciado de *N. paraguayensis* principalmente por apresentar uma abertura bucal triangular, com três lábios simples (em *N. paraguayensis*, a abertura bucal é hexagonal, com três lábios bilobados) e pela presença de papilas somáticas (ausentes em *N. paraguayensis*). Nos machos de *Neocosmocercella bakeri* sp. n., a distribuição de papilas cloacais sésseis também é diferente dos machos de *N. paraguayensis*: um par de papilas sésseis pré-cloacais vs. dois pares; lábio superior da cloaca com dois pares de papilas e uma papila grande não pareada vs. três pares de papilas e uma papila grande não pareada; dois pares de papilas ad-cloacais laterais na nova espécie e ausentes em *N. paraguayensis*; três pares de papilas pós-cloacais, dois pares ventrais e um par de papilas laterais vs. cinco pares, três subventrais e dois laterais. Adicionalmente, o gubernáculo de *Neocosmocercella bakeri* sp. n., é menor (25 – 32) do que o de *N. paraguayensis* (34 – 43). Os úteros nas fêmeas da nova espécie são opistodelfos, e o corpo é menor e dilatado posteriormente (2,4 – 2,6 mm), em *N. paraguayensis* as fêmeas são prodelfas com corpo uniforme em toda a extensão e maiores (3,2 – 4,2 mm).

A distribuição de papilas cloacais sésseis é comumente utilizada para separação de espécies de Cosmocercidae (Baker and Adamson, 1977), desta forma a variação de papilas observada entre *Neocosmocercella bakeri* sp. n. e *N. paraguayensis* associada com outros caracteres morfológicos são suficientes para a descrição desta nova espécie, eliminando a possibilidade de que as papilas sejam uma variação intraespecífica. Assim, variações morfológicas associadas a variações morfométricas dos parasitos encontrados em *P. vaillantii* representam uma nova espécie de *Neocosmocercella*; segunda espécie do gênero e a segunda espécie de cosmocercíneo parasitando este hospedeiro. De acordo com Bursey et al. (2001), *Cosmocercella phyllomedusae* Baker and Vaucher, 1983 foi a primeira espécie de cosmocercíneo a ser registrada como parasito de *P. vaillantii*.

As análises por microscopia eletrônica de varredura, foram de grande importância para confirmar caracteres que diferem a espécie descrita no presente trabalho da espécie-tipo do gênero, revelando com maiores detalhes o padrão de distribuição das papilas, a presença de sésseis, detalhes das papilas vesiculadas, boca com abertura triangular, lábios simples e papilas somáticas presentes nos machos e nas fêmeas. *Neocosmocercella bakeri* sp. n., é a primeira espécie do gênero analisada por microscopia eletrônica de varredura adicionando novos dados morfológicos para o gênero. Este é o primeiro registro de *Neocosmocercella* parasitando anuros da Amazônia brasileira, com descrição da segunda espécie do gênero.

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Legenda das figuras

Figura 1. Desenho de *Neoscomocercella bakeri* n. sp. parasito de *Phyllomedusa vaillantii*. a. Visão geral da fêmea; b. Extremidade anterior da fêmea, visão lateral; c. Visão frontal da extremidade anterior das fêmeas; d. Visão geral do macho; e. Espículos e gubernáculo; f. Vulva, vagina e saco uterino, visão lateral; g. Extremidade posterior do macho, visão lateral; h. Ovo larvado. Escala das barras: a, d, f, g, 200 μm ; b, e, 50 μm ; c, 25 μm ; h, 100 μm .

Figura 2. Microscopia Eletrônica de Varredura de *Neoscomocercella bakeri* n. sp. parasito de *Phyllomedusa vaillantii*. a. Abertura triangular da boca com três lábios simples; b. Extremidade anterior com papilas somáticas; c. Região da vulva; d. Distribuição das papilas vesiculadas na região posterior dos machos; e. Papila vesiculada; f. Distribuição das papilas cloacais; g. Extremidade posterior da fêmea, visão lateral; h. Extremidade posterior da fêmea, visão ventral. Abreviações: asterisco: lábios; Vu: vulva; Setas em cada figura: papila somática; Sp: papila séssil pré-cloacal; Ad: papila ad-cloacal; Pc: papila pre-cloacal; Ps: papilla pós-cloacal. Escala das barras: a, 2 μm ; b, e, 10 μm ; c, 20 μm ; d, 100 μm ; f – h, 50 μm .

ANEXO 1

Systematic Parasitology

Instructions for Authors

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Authorship should incorporate and should be restricted to those who have contributed substantially to the work in one or more of the following categories:

- Conceived of or designed study
- Performed research
- Analyzed data
- Contributed new methods or models
- Wrote the paper

GENERAL

The following types of communication will be considered for publication:

- papers of about 6,000 words (fully illustrated)
- brief communications or research notes (about 2,000 words), not normally illustrated
- major revisions (about 24,000 words), fully illustrated.

Any communication which contains descriptions of new taxa (genera or species) should be accompanied by specimens (preferably paratypes) for scrutiny by the referees and by a statement where the holotypes are deposited. Papers and major revisions should include, at the beginning, a summary (approximately 250 words for papers and 500 words for major revisions).

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Always use footnotes instead of endnotes.

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The authorities and date of all parasite taxa should be given when the names (full binomen) are first cited in the abstract and the text. Subsequently, the generic component is abbreviated except at the beginning of a sentence.

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- This result was later contradicted by Becker and Seligman (1996).
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Reference list entries should be alphabetized by the last names of the first author of each work.

- Journal article
Harris, M., Karper, E., Stacks, G., Hoffman, D., DeNiro, R., Cruz, P., et al. (2001). Writing labs and the Hollywood connection. *Journal of Film Writing*, 44(3), 213–245.
- Article by DOI
Slifka, M. K., & Whitton, J. L. (2000) Clinical implications of dysregulated cytokine production. *Journal of Molecular Medicine*, doi:10.1007/s001090000086
- Book
Calfee, R. C., & Valencia, R. R. (1991). *APA guide to preparing manuscripts for journal publication*. Washington, DC: American Psychological Association.
- Book chapter
O’Neil, J. M., & Egan, J. (1992). Men’s and women’s gender role journeys: Metaphor for healing, transition, and transformation. In B. R. Wainrib (Ed.), *Gender issues across the life cycle* (pp. 107–123). New York: Springer.
- Online document
Abou-Allaban, Y., Dell, M. L., Greenberg, W., Lomax, J., Peteet, J., Torres, M., & Cowell, V. (2006). Religious/spiritual commitments and psychiatric practice. Resource document. American Psychiatric Association. http://www.psych.org/edu/other_res/lib_archives/archives/200604.pdf. Accessed 25 June 2007.

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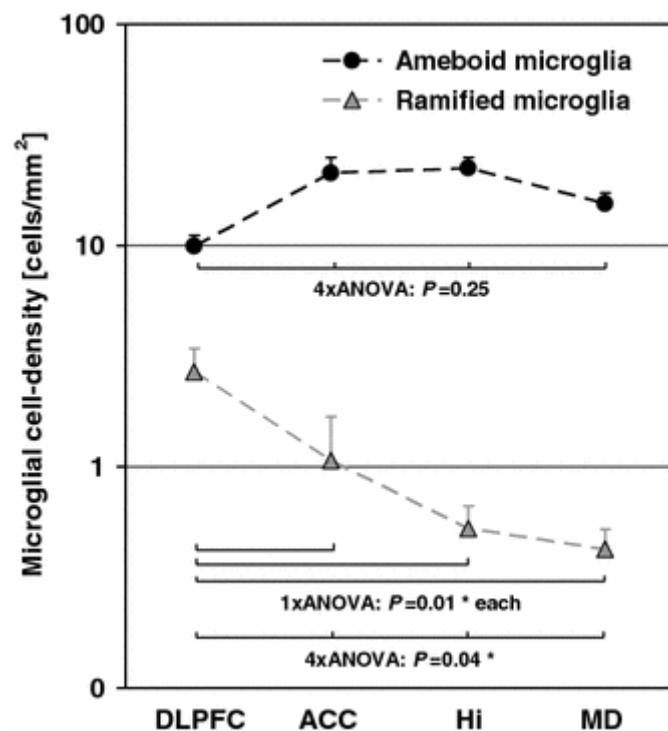
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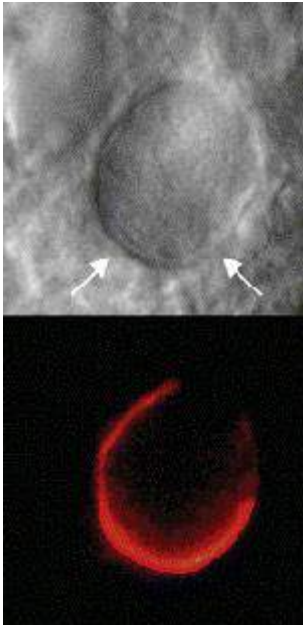
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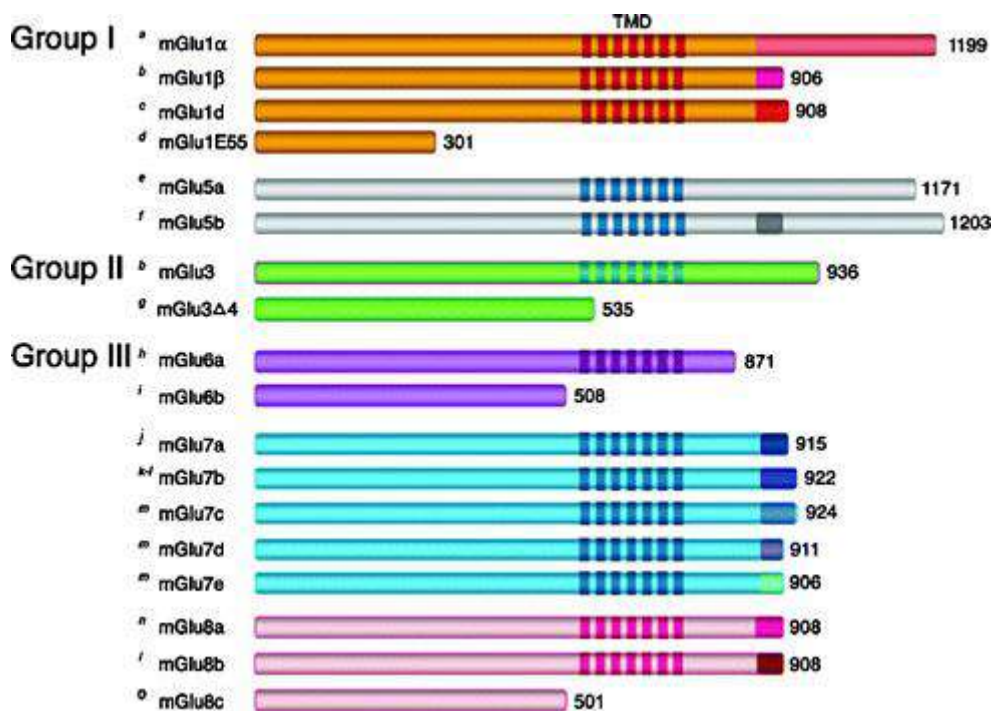
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ANEXO 2

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Biomedical Scientist - Ph.D. in Biology of Infectious and Parasitic Agents

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Date: 2016-11-15 11:10 GMT-03:00

Subject: SYPA-D-16-00101R1 - Ad Hoc from Editor to Author

To: Francisco Tiago Vasconcelos Melo <ftiago@ufpa.br>

Dear Francisco,

I am pleased to confirm that your paper has been accepted for publication in Systematic Parasitology and is now being copyedited. Please feel free to refer to it as 'in press' (2017) in any other communications that you may be preparing.

Best wishes,
Aneta

Aneta Kostadinova
Editor-in-Chief
Systematic Parasitology

ANEXO 3

Systematic Parasitology

New species of *Neocosmocercella* Baker and Vaucher, 1983: a parasite of the large intestine of *Phyllomedusa vaillantii* (Anura: Phyllomedusidae) from the Caxiuanã National Forest, Eastern Amazon, Brazil

--Manuscript Draft--

Manuscript Number:	SYPA-D-16-00101R1									
Full Title:	New species of <i>Neocosmocercella</i> Baker and Vaucher, 1983: a parasite of the large intestine of <i>Phyllomedusa vaillantii</i> (Anura: Phyllomedusidae) from the Caxiuanã National Forest, Eastern Amazon, Brazil									
Article Type:	Original Research Paper									
Keywords:	Nematodes, Cosmoceroidea, <i>Neocosmocercella</i> , Eastern Amazon									
Funding Information:	<table border="1"> <tr> <td>CAPES (Parasitologia Básica 2010)</td> <td>Jeannie Nascimento dos Santos</td> </tr> <tr> <td>Conselho Nacional de Desenvolvimento Científico e Tecnológico (pVe A033_2013)</td> <td>Jeannie Nascimento dos Santos</td> </tr> <tr> <td>Conselho Nacional de Desenvolvimento Científico e Tecnológico (PVE A033_2013)</td> <td>Jeannie Nascimento dos Santos</td> </tr> <tr> <td>Conselho Nacional de Desenvolvimento Científico e Tecnológico (Productivity research scholarship)</td> <td>Jeannie Nascimento dos Santos</td> </tr> </table>	CAPES (Parasitologia Básica 2010)	Jeannie Nascimento dos Santos	Conselho Nacional de Desenvolvimento Científico e Tecnológico (pVe A033_2013)	Jeannie Nascimento dos Santos	Conselho Nacional de Desenvolvimento Científico e Tecnológico (PVE A033_2013)	Jeannie Nascimento dos Santos	Conselho Nacional de Desenvolvimento Científico e Tecnológico (Productivity research scholarship)	Jeannie Nascimento dos Santos	
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Ana Nunes Santos, Bsc.										
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Order of Authors Secondary Information:										

Response to Reviewers:

We are thankful to both reviewers suggestions to improve the manuscript. We appreciate all their suggestions, corrections and comments. All corrections marked in the text of the manuscript.

We will present bellow the detailed replies to comments of reviewer 1 and 2.

Page 2

-Line 6: Please correct "Baker and Vaucher (1983)" to "Baker and Vaucher, 1983". Also please consider that the "&", not "and" should be used before the last taxa authority name, according to the journal format.

R= Okay.

Lines 12-13: I suggest deleting the sentence concerning SEM from the Abstract. Instead, I would recommend mentioning in the Abstract the amended diagnosis of Neocosmocercella.

R= Done.

Page 3

I suggest including into the Material and Methods section the information on the license permission, the description of the procedure of hosts' euthanasia, and the method of nematode measurements taking.

R= We included the information required.

Lines 19-20: "it is the only monotypic genus". To the best of my knowledge, there are other monotypic genera in Cosmocercidae, e.g., Austraplectana, Blanusia, Neosomatiana, Paraleptonema, etc.

R= We removed this information according to the suggestions.

Lines 12 and 24: "genera parasitizing". I would suggest replacing with "species of the genera parasitizing".

R= Replaced.

Lines 48-49: Please correct "Allotypes, holotypes, and paratypes" to "Holotype, allotype and paratypes of the new species".

R= Corrected

Page 4

Line 12: Please correct "1.227" to "1,227" or "1227".

R= Done

Lines 16-17: I suggest omitting "Muséum national d'Histoire naturelle, Paris".

R= Omitted

Lines 30-31: Please correct "an anterior" to "anterior".

R= Corrected.

Lines 33-34: Please correct "opistodelphic" to "opisthodelphic", since the latter form is used throughout the text.

R= We corrected

Line 41: Please correct "length and 38 - 48 (44) width" to "long and 38 - 48 (44) wide".

R= Modified as required.

Lines 42-43, 58-59: "at esophagus-intestine region". Did you mean "at esophagus-intestine junction"?

R= we replaced

Line 44: "papillae ... which becomes gradually smaller". Please correct the grammar.

R= Corrected

Lines 45-46: "in left side", "on the right side". Please correct to "on left side" and "on right side".

R= Corrected

Lines 56-57: Please add "in" before "width".

Added

Lines 59-60: Please correct "pre equatorial" to "pre-equatorial".
R=corrected.

Page 5

Line 0: Please correct "e" to "and".

R=replaced

Lines 4-5: Please correct "length, with a long and sharply point" to "long, sharply pointed".

R= corrected.

Line 9: Please replace "identified" with "erected".

R= replaced

Lines 15-16: Please replace "at" with "on" or "in".

R= We replaced

Lines 25-26: I suggest avoiding phylogenetic terms ("apomorphies", "synapomorphy"), since the phylogenetic analysis was not performed. The characters discussed are merely either diagnostic or not diagnostic for particular taxa.

R= We replaced the terms.

Lines 42-43: "The females of Neocosmocercella sp. n. are opisthodelphic ...". Please correct mistakes in the sentence. Not females, but the uteri are opisthodelphic or prodelphic.

R= Done

Lines 57-58: Please correct "of subfamily" to "of the subfamily".

R= corrected.

Figure captions

Please remove articles ("a", "the") before "male" and "female".

R= removed

Line 12: Please replace "Detail" with "Region".

R= Replaced

Lines 13-14: Please erase "Detail of the".

R= Erased

Lines 18-19: Please provide correct explanation to all scale bars.

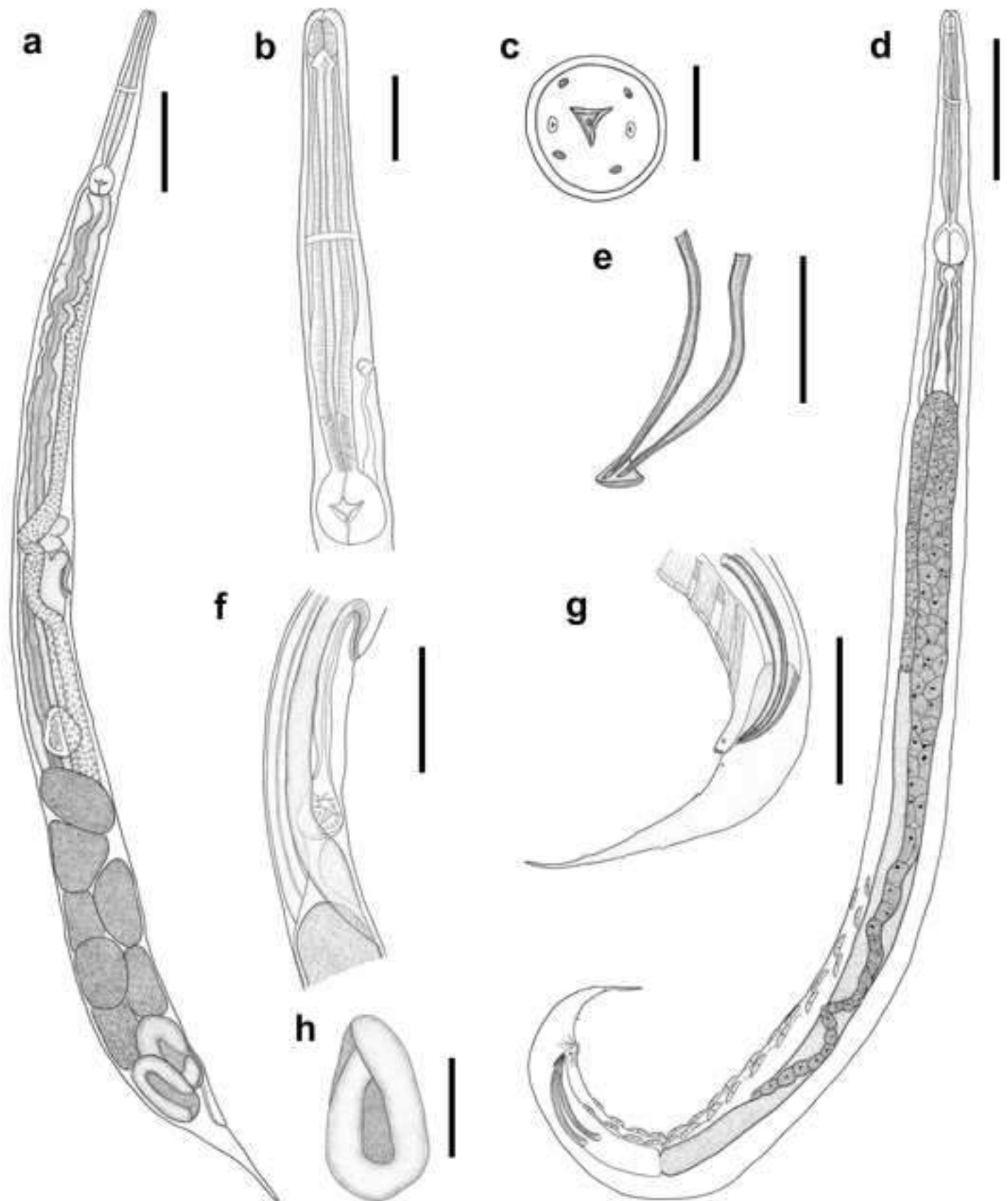
R= We added all the information about scale bars.

Reviewer #2: The article has importance in the area related with nematode parasites of amphibians, specially because it describes a new species, and additionally because *Phyllomedusa vaillantii* is a poorly studied amphibian host.

The bibliography is right for a scientific article and it is updated, and the methodology used is appropriate.

Minor comments related to the English language and format are made in the manuscript. See attachment

R= We accepted all modifications suggested by the reviewer. Somewhere also suggested by the reviewer #1.





1 **New species of *Neocosmocercella* Baker and Vaucher, 1983: a parasite of the large intestine of**
2 ***Phyllomedusa vaillantii* (Anura: Phyllomedusidae) from the Caxiuanã National Forest, Eastern Amazon,**
3 **Brazil**

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6 Ana Nunes dos Santos¹, Allan Rodrigo Oliveira Rodrigues¹, Jeannie Nascimento dos Santos¹, Cynthya
7 Elizabeth González², Francisco Tiago de Vasconcelos Melo¹

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22 Helmintologia “Profa Dra Reinalda Marisa Lanfredi” Instituto de Ciências Biológicas - Universidade Federal do
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Abstract

Neocosmocercella bakeri sp. n. is a parasite of the large intestine of *Phyllomedusa vaillantii* Boulenger found in the Caxiuanã National Forest in the Eastern Brazilian Amazon. The new species is easily distinguished from the type species of the genus, *Neocosmocercella paraguayensis* Baker & Vaucher 1983 by presenting a triangular mouth opening with three simple lips, in contrast to the three bilobed lips and hexagonal mouth opening in *N. paraguayensis*. In addition, the new species has somatic papillae, which are absent in *N. paraguayensis*. The males are distinguished by the distribution of sessile cloacal papillae and the dimensions of the gubernaculum, whereas the females are distinguished by their smaller size and opisthodelphic uterus. Beyond the second species of the genus, we are discussing about taxonomic characters on diagnosis of *Neocosmocercella*. This study is the first to report the infection of anurans with *Neocosmocercella* in the Brazilian Amazon, the record of a new host for the genus, and the description of the second species of the genus.

Introduction

Phyllomedusa Wagler is a genus of anurans of the family Phyllomedusidae, containing 16 species, and occurs from Central America to South America, including Trinidad, to northern Argentina and Uruguay (Frost 2016). *Phyllomedusa vaillantii* Boulenger is nocturnal and usually found in leaf litter and shrubs near streams or permanent bodies of water in tropical forests (Duellman 1978). In South America, it occurs from the Guianas and Amazon Basin of Guyana, Suriname, French Guiana, and northeastern Brazil to Colombia, Peru, northern Bolivia, state of Amazonas, and Venezuela (Frost 2016).

To date, species of four nematode genera have been found parasitizing *Phyllomedusa* spp.: *Cosmocercella* Steiner, 1924; *Physalopteroides* Wu & Liu, 1940; *Oswaldocruzia* Travassos, 1917; and *Cosmocerca* Diesing, 1861 (Campião et al. 2014). The nematode genera *Cosmocercella* and *Cosmocerca* belong to the family Cosmocercidae and are commonly found parasitizing the intestines of amphibians but are rarely found in reptiles (Vicente et al. 1990). Among the genera of the Cosmocercidae family, *Neocosmocercella* Baker & Vaucher, 1983 is prominent because it is a monotypic genus, and the species *Neocosmocercella paraguayensis* Baker & Vaucher, 1983 has been found parasitizing *Pithecopus hypochondryalis* (Daudin) (*Phyllomedusa hypochondrialis*) in Paraguay (Baker and Vaucher 1983).

Only species of three nematode genera have been recorded parasitizing *Phyllomedusa vaillantii*: *Cosmocercella*, *Physalopteroides*, and *Oswaldocruzia* (Campião et al. 2014). This study describes a new species of *Neocosmocercella* and reports the parasitism of this species in *P. vaillantii* in the Eastern Brazilian Amazon.

Materials and Methods

Ten specimens of *Phyllomedusa vaillantii* were collected in the Ferreira Pena Scientific Station (host specimens were collected under permits 0004/06 NUC SUPES PA and SISBIO 32660-1), located in the Caxiuanã National Forest, in the municipality of Melgaço, state of Pará, Brazil, during an expedition conducted in March 2015 for the collection of helminths that parasitize amphibians and reptiles. The hosts were euthanised by injection of lidocaine hydrochloride 2%, and their internal organs were examined under a dissecting scope. Helminths were collected, transferred to saline, and fixed in 70% ethanol, and for morphological and morphometric analysis, the nematodes were cleared in Amman's lactophenol and analyzed using an Olympus BX41 microscope (Olympus, Tokyo, Japan) coupled to a drawing tube. The measurements are given in micrometers unless otherwise indicated and are presented as the range followed by the mean in parentheses.

For scanning electron microscopy, samples were post-fixed in 1% O₂OS₄, subjected to dehydration in increasing ethanol concentrations until critical point drying using CO₂, metallized in gold, and examined using a Vega3 microscope (TESCAN) in the Laboratory of Embryology and Histology of the Federal Rural University of Amazonia (Universidade Federal Rural da Amazônia–UFRA).

Holotypes, allotypes and paratypes of the new species were deposited in the Invertebrate Collection of the Emílio Goeldi Museum (Museu Paraense Emílio Goeldi–MPEG), and the hosts were deposited in the herpetological collection of MPEG.

Order Ascaridida Skrjabin and Shulz, 1940

Superfamily Cosmocercidae Travassos, 1925

1 **Family Cosmocercidae Travassos, 1925**

2
3 ***Neocosmocercella* Baker and Vaucher, 1983**

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6 *Neocosmocercella bakeri* sp. n.

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9 *Type-host:* *Phyllomedusa vaillantii* Boulenger, 1882

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11 *Infection site:* Intestine

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13 *Type-locality:* Caxiuanã National Forest (1°47'N 51°26'02.5"W), Pará, Brazil.

14
15 *Prevalence:* 30% (3 of 10)

16
17 *Mean intensity:* 409 per infected host (range 5 – 1227)

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19 *Mean abundance:* 129.3 per host

20
21 *Type-material:* Holotype (MPEG 0068); Allotype (MPEG 0069); Paratype(MPEG 0070)

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23 *Etymology:* The new species is named after Michael R. Baker in recognition of his many contributions on the systematics and biology of parasites of amphibians and reptiles.

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17 Description (Figs. 1, 2)

19 *General description:* small, slender nematodes. Sexual dimorphism evident, females larger than males, males with curved caudal region (Fig. 1a, d). Delicate transversal cuticle striations; lateral alae present in both sexes, beginning at esophageal bulb extending to cloacal region in males and after anus region in females. Mouth triangular, with three lips; dorsal lip with two cephalic papillae and each ventrolateral lip with one cephalic papilla and one large amphid (Fig. 1c, 2a). Esophagus divided into anterior pharyngeal portion, corpus, isthmus and large bulb with chitinous valves (Fig. 1b). Nerve ring situated at esophagus mid region, excretory pore close to esophageal bulb (Fig. 1b). Didelphic, opisthodelphic females. Somatic papillae present (Fig. 2b, g, h). Tail slender and sharply pointed in both sexes (Fig. 1g, 2g, h).

28 *Males (based on 1 holotype and 9 paratypes):* Total length 1.4 – 2.06 (1.6) mm, esophagus 302.5 – 354.5 (341), length including bulb, pharynx 23 – 30 (27), corpus 204 – 253 (228), isthmus 26 – 43 (33), bulb 47 – 52 (50) long and 38 – 48 (44) wide. Nerve ring 127 – 148 (139) and excretory pore 200 – 221 (207) from anterior end. Body width at esophagus-intestine junction 45 – 66 (50). Posterior end ventrally curved. Precloacal region with two ventral rows of 25 to 29 vesiculated papillae (Fig. 2e) becoming gradually smaller, varying between 11 to 14 on left side and 12 to 15 on right side (Fig. 1d, g, 2d). One pair of sessile precloacal papilla. Anterior lip of cloaca with two pairs of paired papillae and a large unpaired superior papilla; two pairs adcloacal and a lateral pair (Fig. 2f). Spicules subequal 104 – 139 (113) long (Fig. 1e), triangular gubernaculum concave, 25 – 32 (31). Tail slender, sharply pointed, 126 – 148 (137) long.

38 *Females (based on 1 allotype and 9 paratypes):* Body starting slender and dilating posteriorly, 2.4 – 2.6 (2.5) mm long, width at vulva 128 – 184 (154). Esophagus 403 – 451 (423) in length including bulb; pharynx 29 – 37 (33), corpus 267 – 304 (285), isthmus 35 – 45 (39), bulb 59 – 77 (65) in length and 56 – 67 (60) in width. Nerve

ring 139 – 186 (166) and excretory pore 210 – 288 (265) from anterior end. Body in width at esophagus-intestine junction 91 – 115 (99). Vulva discreet, pre-equatorial (Fig. 1f, 2c), situated at 1.02 – 1.14 (1.08) mm from anterior end. Vagina well developed, directed anteriorly (*vagina vera*) and folding back posteriorly (*vagina uterina*) ending at muscular uterine sac (Fig. 1f), ovoviviparous (Fig. 1a). Uteri filled with 8 to 10 eggs, last eggs with larvae (Figs. 1a, h), eggs in morula 152 – 185 (168) long and 96 – 135 (114) width. Tail slender 224 – 277 (241) long, sharply pointed.

Discussion

The genus *Neocosmocercella* was erected by Baker and Vaucher (1983) and was described using specimens found in the large intestine of *Pithecopus hypochondrialis* (Daudin) (*Phyllomedusa hypochondrialis*) in Paraguay. Baker and Vaucher (1983) descriptions were primarily based on individual characteristics, including the presence of a uterine sac, elongated vagina and, in males, a coiled posterior region and vesiculated papillae on posterior region. Moreover, aspects related to the mouth opening and position of the uterus were reported as important generic characters: hexagonal mouth opening, formed by three bilobed lips, prodelphic uterus and the uterine sac (Baker and Vaucher 1983); all of these characteristics were corroborated by Draghi et al. (2015) in specimens of *N. paraguayensis* found in *Pithecopus azureus* (Cope) (*Phyllomedusa azurea*) in Formosa, Argentina. However, among these generic characteristics, *Neocosmocercella bakeri* sp. n. has only pre-cloacal vesiculated papillae in males and an elongated vagina and uterine sac in females; the species described in this study has a triangular mouth opening, simple lips, and an opisthodelphic uterus. Therefore, these characteristics may be diagnostic only for *N. paraguayensis* and not a diagnostic character of the genus, as proposed by these authors.

Neocosmocercella bakeri sp. n. is easily distinguished from *N. paraguayensis* primarily by the presence of a triangular mouth opening, with three simple lips (in *N. paraguayensis*, the mouth opening is hexagonal, with three bilobed lips) and the presence of somatic papillae (absent in *N. paraguayensis*). The distribution of sessile cloacal papillae in males of *Neocosmocercella bakeri* sp. n. is markedly different from those in males of *N. paraguayensis*: one pair of pre-cloacal sessile papillae vs. two pairs; the upper lip of the cloaca has two pairs of papillae and a large unpaired papillae vs. three pairs of papillae and one large unpaired papilla; two pairs of lateral ad-cloacal papillae in the new species and absence of these papillae in *N. paraguayensis*; and three pairs of post-cloacal papillae (two ventral pairs and one lateral pair) vs. five pairs of papillae (three subventral pairs and two lateral pairs). In the same way, the gubernaculum of *Neocosmocercella bakeri* sp. n. is smaller (24.6 – 32.4) than that of *N. paraguayensis* (34 – 43). The uteri on females of the new species are opisthodelphic, and the body is smaller and dilated posteriorly (2.4 – 2.6 mm); the females of *N. paraguayensis* are prodelphic, and the body is larger and uniform throughout its length (3.2 – 4.2 mm).

The distribution and shape of the ornate papillae are the main characteristics used for separation of the genera of the subfamily Cosmocercinae (Anderson et al. 2009). Therefore, the nematodes found parasitizing the large intestine of *Phyllomedusa vaillantii* in this study can be classified in the genus *Neocosmocercella* because the male specimens only present vesiculated papillae in the posterior region. The genus *Cosmocercella* Steiner, 1924 is morphologically similar to *Neocosmocercella*, as they present this type of papillae; however, the former is easily differentiated by having rosette papillae in the posterior region (Baker and Adamson 1977). Additionally *Neocosmocercella bakeri* sp. n. differs from species of *Cosmocercella* and of species from other

1 genera of the subfamily Cosmocercinae by presenting an elongated uterine vagina and uterine sac in females
2 (absent in the other genera).

3 The distribution of sessile cloacal papillae is widely used to separate species of Cosmocercidae (Baker
4 and Adamson 1977); therefore, the variation in papillae observed between *Neocosmocercella bakeri* sp. n. and
5 *N. paraguayensis* associated with other morphological characteristics are sufficient for the proposal of a new
6 species, eliminating the possibility of intraspecific variation in the papillae. Thus, morphological variations
7 combined with morphometric variations in the parasites found in *P. vaillantii* indicate the presence of a new
8 species of *Neocosmocercella*; this is the second species of the genus and the second species of Cosmocercinae
9 found parasitizing this host. Bursey et al. (2001) reported that *Cosmocercella phyllomedusae* Baker and
10 Vaucher, 1983 was the first species of Cosmocercinae found parasitizing *P. vaillantii*. The scanning electron
11 microscopy analyses were essential to confirm the characteristics that distinguish this species from the type
12 species of the genus, revealing in greater detail the pattern of distribution of sessile papillae, vesiculated
13 papillae, mouth with triangular opening, simple lips, and somatic papillae in males and females.
14 *Neocosmocercella bakeri* sp. n. is the first species of the genus analyzed by scanning electron microscopy,
15 generating new morphological data for the genus. This study is the first to record the infection of anurans with
16 *Neocosmocercella* in the Brazilian Amazon and describes the second species of the genus.

17 18 **Acknowledgments**

19 We are grateful to the field team and to Dr. Elane Giese for granting access to the Laboratory of Animal
20 Embryology and Histology of the Federal Rural University of Amazonia and for scanning electron microscopy
21 analyses.

22 23 **Compliance with Ethical Standards**

24 All applicable institutional, national and international guidelines for the care and use of animals were followed.
25 Specimens were collected under permits 0004/06 NUC SUPES PA, project “Biodiversity survey of the
26 herpetofauna according to the Research Program on PPBIO eastern Amazonia” and SISBIO 32660-1, project
27 “Amphibian and reptile diversity and associated helminth parasites in the Amazon region”

28 29 **Funding:**

30 The financial support for this study was provided by PVE A013/2013 (CAPES/CNPq); ‘Parasitologia Básica
31 2010’ (CAPES); PIBIC/CNPQ; Productivity Scholarship (CNPQ) to J.N.S.; and PROPESP/FADESP under
32 research proposal PAPQ2016.

33 34 **Conflict of interest:**

35 The authors declare that they have no conflict of interest

36 **Ethical approval:**

37 All procedures performed in studies involving animals were in accordance with the ethical standards of the
38 institution or practice at which the studies were conducted

1
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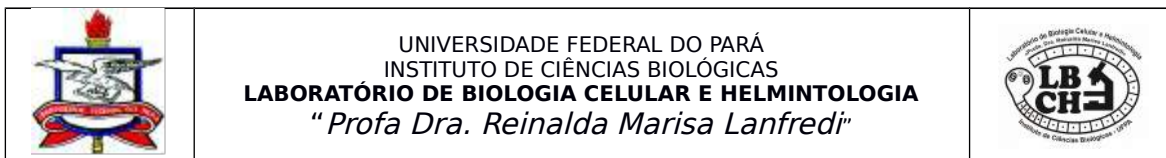
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2 **Figure captions**

3 **Fig. 1 – Line drawings of *Neoscomocercella bakeri* n. sp. parasite of *Phyllomedusa vaillantii*.** a. General
4 structure of female; b. Anterior end of female, lateral view; c. Frontal view of the anterior end of female; d.
5 General view of male; e. Spicules and gubernaculum; f. Vulva, vagina and uterine sac, lateral view; g. Posterior
6 end of male, lateral view; h. Larvated egg. Scale bars: a, d, f, g, 200 μm ; b, e, 50 μm ; c, 25 μm ; h, 100 μm .

7 **Fig. 2 – Scanning Electron Microscopy of *Neoscomocercella bakeri* n. sp. parasite of *Phyllomedusa***
8 ***vaillantii*.** a. Triangular mouth opening with three simple lips; b. Anterior end with somatic papillae; c. Region
9 of vulva; d. Distribution of vesiculated papillae in the posterior region in males; e. Vesiculated papillae; f.
10 Distribution of cloacal papillae; g. Posterior end of female, lateral view; h. Posterior end of female, ventral view.
11 Abbreviations: asterisk: lips; Vu: vulva; Arrows in each figure: somatic papillae; Sp: sessil precloacal papillae;
12 Ad: adcloacal papillae; Pc: precloacal papillae; Ps: postcloacal. Scale bars: a, 2 μm ; b, e, 10 μm ; c, 20 μm ; d,
13 100 μm ; f – h, 50 μm .

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SUBMISSION OF A MANUSCRIPT

SEP, 2016

Dear Aneta Kostadinova
Editor-in-Chief
Systematic Parasitology

I am pleased to submit an original research article titled " **New species of *Neocosmocercella* Baker and Vaucher, 1983: a parasite of the large intestine of *Phyllomedusa vaillantii* (Anura: Phyllomedusidae) from the Caxiuanã National Forest, Eastern Amazon, Brazil.**" for publication in **Systematic Parasitology**.

We believe that this manuscript is appropriate for publication in your journal because it presents novel information on systematic and morphology of species of Cosmocercidae using light and scanning electron microscopy.

We identified for the first time, the second species of the genus *Neocosmocercella*. This species was identified thirty-three years after type-species, and the new species has unique and important morphological characters. Then, this work is likely to be of broad interest to the readership of **Systematic Parasitology** because of the wide relevance of parasitic nematodes in general and because our findings will be helpful in identifying this species of nematode.

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose. All authors have read and approved the final version of the manuscript. Thank you for your consideration, and we look forward to hearing from you at your earliest convenience.

The paper was edited for proper English language, grammar, punctuation, spelling and overall style by the editors of American Journal Experts (see the certificate below).



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EDITORIAL CERTIFICATE

This document certifies that the manuscript listed below was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at American Journal Experts.

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New species of Neocosmocercella Baker and Vaucher, 1983: a parasite of the large intestine of Phyllomedusa vaillantii (Anura: Phyllomedusidae) from the Caxiuanã National Forest, Eastern Amazon, Brazil.

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