UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA COMPARADA

GABRIELA NARDI

ANÁLISE TAXONÔMICA E MOLECULAR DE POPULAÇÕES DE *Neoplecostomus* (TELEOSTEI: SILURIFORMES) DA DRENAGEM DO ALTO RIO PARANÁ

Maringá, PR
2019

UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA COMPARADA

GABRIELA NARDI

ANÁLISE TAXONÔMICA E MOLECULAR DE POPULAÇÕES DE *Neoplecostomus* (TELEOSTEI: SILURIFORMES) DA DRENAGEM DO ALTO RIO PARANÁ

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Biologia Comparada, como parte dos requisitos para obtenção do título de Mestra em Biologia das Interações Orgânicas.

Orientador: Prof. Dr. Cláudio Henrique Zawadzki
Coorientador: Dr. Fábio Fernandes Roxo

Maringá, PR
2019

Dados Internacionais de Catalogação na Publicação (CIP)
(Biblioteca Central - UEM, Maringá, PR, Brasil)

Nardi, Gabriela
N223a Análise taxonômica e molecular de populações de *Neoplecostomus* (Teleostei: Siluriformes) da drenagem do Alto Rio Paraná/ Gabriela Nardi. -- Maringá, 2019.
43 f. : il. (algumas color.)

Orientador (a): Prof. Dr. Cláudio Henrique Zawadzki.
Coorientador (a): Prof. Dr. Fábio Fernandes Roxo.
Dissertação (Mestrado) – Universidade Estadual de Maringá, Centro de Ciências Biológicas, Programa de Pós-Graduação em Biologia Comparada, 2019.

1. Cascudos neotropicais. 2. DNA *barcode*. 3. Neoplecostomini. 4. Taxonomia integrativa. I. Zawadzki, Cláudio Henrique, orient. II. Roxo, Fábio Fernandes, coorient. III. Universidade Estadual de Maringá. Centro de Ciências Biológicas. Programa de Pós-Graduação em Biologia Comparada. IV. Título.

CDD 21.ed. 597.4

MAS-CRB 9/1094

FOLHA DE APROVAÇÃO

GABRIELA NARDI

ANÁLISE TAXONÔMICA E MOLECULAR DE POPULAÇÕES DE *Neoplecostomus* (TELEOSTEI: SILURIFORMES) DA DRENAGEM DO ALTO RIO PARANÁ

Dissertação apresentada ao Programa de Pós-Graduação em Biologia Comparada do Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Mestre em Biologia das Interações Orgânicas pela Comissão Julgadora composta pelos membros:

COMISSÃO JULGADORA

Prof. Dr. Claudio Henrique Zawadzki
Universidade Estadual de Maringá (Presidente)

Prof. Dr. Weferson Júnio da Graça
Universidade Estadual de Maringá

Prof. Dr. Bruno Francelino de Melo
Universidade Estadual Paulista

Aprovada em: 22 de fevereiro de 2019.
Local de defesa: Bloco H-90, Auditório, *campus* da Universidade Estadual de Maringá.

AGRADECIMENTOS

Agradeço ao meu orientador Cláudio Henrique Zawadzki pela oportunidade e confiança a mim concedida para a realização deste trabalho, que me proporcionou grandes aprendizados e amizades que levarei sempre comigo. Agradeço ainda pela paciência, ajuda, conversas e por estar sempre disposto a atender quaisquer questionamentos.

Aos meus pais, Lucia Nascimento Nardi e José Roberto Nardi por tudo que fazem por mim, especialmente por sempre me apoiaram nas decisões e caminhos que opto por seguir, além do incentivo da vida de sempre seguir em frente (daquele café quentinho que sempre aparece quando menos espero).

Às minhas irmãs Roberta e Rafaela Nardi, mulheres que tanto amo e admiro, por estarem sempre comigo (mesmo que a vários quilômetros de distância), por todo companheirismo, por proporcionarem muitos momentos agradáveis e engraçados e simplesmente por serem pessoas com quem posso sempre contar.

Aos meus amigos de laboratório, Gabriel (Gabera), Augusto (Bigode), Filipe (Nego), Renata (Rubs) Ota, Rianne e Renan, por me receberem e aguentarem nestes dois anos, pelas diversas brincadeira e risadas, além de sempre estarem a disposição para ajudar uns aos outros, tudo isso torna o ambiente de trabalho muito mais agradável, o que é de suma importância. Agradeço ainda, a Carla Simone Pavanelli e Weferson Júnio da Graça por se estarem disponíveis para conversas, questionamentos e discussões (e no caso de Weferson também por ensinar que o café nunca está forte o suficiente).

Ao meu companheiro de laboratório, de turma de mestrado e "cascudeiro" Iago de Souza Penido que depois desse período trabalhando e estudando juntos e diversos momentos de incríveis conversas e palhaçadas, descobri uma pessoa ímpar e um grande amigo para levar para a vida toda.

Ao pessoal do Laboratório de Genética de Peixes da UNESP de Botucatu que tive o prazer de conhecer, que me receberam e se mostraram a disposição para ajudar tanto no período que estive em Botucatu quanto posteriormente, especialmente à Camila Souza por toda ajuda, atenção e paciência ao me ensinar as técnicas de laboratório.

Ao meu coorientador Fábio Fernandes Roxo (Fio) pela ajuda, aprendizado e atenção que me forneceu sempre que precisei, além das risadas (que foram várias).

À Nadayca (Naná), Mariana (Magrela), Bel Soares e Isa Moraes que me acolheram sob seus tetos no período que passei em Botucatu, seres humaninhos que sou muito grata por ter conhecido e convivido.

À CAPES pela concessão da bolsa nestes anos.

Àqueles que por algum motivo não foram citados mais que contribuíram para que este trabalho fosse realizado.

Análise taxonômica e molecular de populações de *Neoplecostomus* (Teleostei: Siluriformes) da drenagem do alto rio Paraná

RESUMO

Loricariidae é a família mais rica em espécies em Siluriformes com 983 espécies válidas. Dentro de Loricariidae, Hypoptopomatinae é composta pelas tribos Otothyrini, Neoplecostomini e Hypoptopomatini. Neoplecostomini é representada por cascudos de pequeno porte restritos ao Escudo Brasileiro. *Neoplecostomus* inclui atualmente 18 espécies válidas, com diversas espécies descritas com integração de dados morfológicos e moleculares. Desta forma, o presente trabalho descreve duas novas espécies de *Neoplecostomus* para a drenagem do alto rio Paraná, estado do Paraná, especificamente para as bacias do rio Piquiri e Ivaí, *Neoplecostomus* sp. 1 e *Neoplecostomus* sp. 2, respectivamente. As novas espécies são diferenciadas de suas congêneres por morfometria, caracteres merísticos e por sequência de DNA do gene mitochondrial citocromo c oxidase subunidade I (COI). Caracteres morfométricos e merísticos foram tomados conforme metodologia padrão para o grupo, e sequências nucleotídicas de 68 espécimes de *Neoplecostomus* com 531 pb foram analisadas. A matriz foi utilizada para calcular a distância genética entre as espécies no programa MEGA 6.0 bem como para fazer análise de *maximum-likelihood* usando RAxML HPC2 on XSEDE incorporado no portal CIPRES. Os suportes dos ramos da topologia obtida foram testados utilizando método estatístico de *bootstrap* com 1.000 pseudoréplicas. Morfologicamente, ambas as espécies diferem da maioria das congêneres por apresentar nadadeira adiposa pouco desenvolvida. Adicionalmente, *Neoplecostomus* sp. 1 apresenta escudo torácico com sua porção anterior reta e iniciando próximo às aberturas branquiais e região nua ao redor da abertura urogenital mais estreita devido às primeiras placas ventrais. *Neoplecostomus* sp. 2 apresenta cabeça arredondada em vista lateral devido ao mesetmoide ser mais proeminente, diferencia-se também por apresentar maior diâmetro orbital/comprimento da cabeça. Geneticamente, os resultados separaram as espécies em questão das demais congêneres, apresentando diferentes níveis de divergência genética, com *Neoplecostomus* sp. 1 formando um clado separado, enquanto *Neoplecostomus* sp. 2 é relacionado com *N. canastra* + *N. doceencis*.

Palavras-chave: cascudos neotropicais, DNA *barcode*, Neoplecostomini, taxonomia integrativa.

Taxonomic and molecular analysis of populations of *Neoplecostomus* (Teleostei: Siluriformes) from upper rio Paraná system

ABSTRACT

Loricariidae is the most species-rich family of Siluriformes with 983 valid species. In Loricariidae, Hypoptopomatinae is composed by the tribes Otothyrini, Neoplecostomini e Hypoptopomatini. Neoplecostomini is represented by small-sized catfishes, restricted to the Brazilian Shield. *Neoplecostomus* currently includes 18 valid species, which several of them were described with integration of morphological and molecular data. Therefore, the present work describes two new species of *Neoplecostomus* from upper rio Paraná drainage, Paraná state, specifically from rio Piquiri and Ivaí basins, *Neoplecostomus* sp. 1 and *Neoplecostomus* sp. 2, respectively. The new species are differentiated from its congeners by morphometry, meristic characters and DNA sequences of cytocrome c oxidase subunit I (COI) mitochondrial marker. Measurements and counts were taken as standard methodology for the group, and nucleotide sequences of 68 specimens of *Neoplecostomus* with 531 pb were analyzed. The matrix was used to calculate the genetic distance between species in MEGA 6.0 and analysis of maximum-likelihood using RAxML HPC2 on XSEDE implemented on the CIPRES portal. The support of each node was tested using 1.000 bootstrap pseudoreplicates. Morphologically, both species differ from most congeners by having ill-developed adipose fin. Additionally, *Neoplecostomus* sp. 1 presents thoracic shield with its anterior portion straight and beginning next to the gill openings, and the naked area around urogenital opening narrower than in other species due to the first ventral plates. *Neoplecostomus* sp. 2 presents a round-shaped head in lateral view due to the pronounced mesethmoid, also differs from the congeners by having greater orbital diameter/head length. Genetically, the molecular results separated the species studied herein from the other congeners, showing different levels of genetic divergence between the clusters, with *Neoplecostomus* sp. 1 forming a separated clade, while *Neoplecostomus* sp. 2 is related to *N. canastra* + *N. doceensis*.

Key words: DNA barcode, Neoplecostomini, Neotropical catfishes, integrative taxonomy.

SUMÁRIO

Introdução geral.....10

Referências.....12

TAXONOMIC AND MOLECULAR ANALYSIS OF POPULATIONS OF *Neoplecostomus* (TELEOSTEI: SILURIFORMES) FROM UPPER RIO PARANÁ SYSTEM

Introduction.....16

Material and Methods…….…….….18

Morphological analysis…..……..…..18

Genetic analysis……...18

DNA sequences and phylogenetic analyses……19

Results……..…23

Discussion……...37

Comparative materials……..38

References……...40

INTRODUÇÃO GERAL

Loricariidae é atualmente a maior família em Siluriformes com 983 espécies válidas (Fricke *et al*., 2019), sendo destas, 230 descritas nos últimos dez anos. Segundo Reis *et al*. (2003) os loricarídeos são distribuídos por toda a região Neotropical, desde o norte da Costa Rica ao sul da Argentina. Estes peixes possuem como principais características o corpo coberto por placas ósseas, boca ventral, com ou sem barbilhões e lábio ventral com papilas (Nelson, 2006). Hipóteses filogenéticas quanto às relações de loricariídeos baseadas em dados morfológicos e moleculares foram propostas por vários autores ao longo do tempo, desde os trabalhos de Eigenmann e Eigenmann (1890), com destaque para os trabalhos morfológicos de Howes (1983), Schaefer (1987), Armbruster (2004) e Pereira e Reis (2017), e moleculares de Montoya-Burgos *et al*. (1998), Chiachio *et al.* (2008), Roxo *et al*. (2014), Lujan *et al*. (2015) e Silva *et al*. (2016) para classificação do grupo.

Schaefer (1987) reconheceu seis subfamílias para Loricariidae: Lithogeneinae, Neoplecostominae, Hypoptopomatinae, Loricariinae, Hypostominae e Ancistrinae. Armbruster (2004) baseado em uma análise usando caracteres morfológicos incluiu esta última subfamília (i.e. Ancistrinae) como parte de Hypostominae, propondo sua classificação como tribo Anscistrini. Posteriormente, Reis *et al*. (2006) descreveram Delturinae baseados em hipóteses prévias de que *Hemipsilichthys gobio*, *H. papillatus*, *H. nimius* e as espécies de *Delturus* apresentavam uma posição mais basal dentro de Loricariidae, como grupo irmão de todas as outras espécies da família com exceção de Lithogeninae. Posteriormente, Chiachio *et al*. (2008) baseados em análises moleculares, propuseram Otothyrinae (antes reconhecida como tribo) como grupo irmão de Neoplecostominae. Mais recentemente, Lujan *et al*. (2015) baseados também em caracteres moleculares, propõem seis subfamílias para Loricariidae: Hypostominae, Hypoptopomatinae, Loricariinae, Rhinelepinae, Delturinae e Lithogeninae com a classificação das tribos Neoplecostomini, Otothyrini e Hypoptopomatini como parte de Hypoptopomatinae.

Regan (1904) propôs Neoplecostominae para alocar *Neoplecostomus granosus* (Cuvier & Valenciennes, 1840). Tal subfamília é composta por espécies de pequeno porte. Sua distribuição esta atualmente restrita às drenagens do escudo brasileiro, incluindo a maior parte das drenagens costeiras atlânticas do rio Maquiné, no sul do Brasil até o rio Paraguaçu, na Bahia, no Nordeste do Brasil e drenagens interiores Jacui,

Uruguai, Iguaçu, Paraná e São Francisco (Pereira & Reis, 2017). Gosline (1945) manteve apenas *Neoplecostomus* na subfamília, mas o mesmo autor, em 1947, considerou o grupo mais abrangente e abrigando doze gêneros: *Neoplecostomus*, *Upsilodus*, *Hemipsilichthys*, *Pareiorhaphis*, *Pareiorhina*, *Kronichthys*, *Corymbophanes*, *Delturus*, *Rhinelepis*, *Canthopomus*, *Pogopoma* e *Pogonopomoides* (Pereira & Reis, 2017). Diversas outras análises filogenéticas utilizando caracteres morfológicos foram realizadas para os membros da subfamília, sendo a mais recente, de Pereira e Reis (2017), que propõe que Neoplecostominae é formada por sete gêneros: *Neoplecostomus, Hirtella, Isbrueckerichthys, Kronichthys, Pareiorhaphis, Pareiorhina* e *Euryochus*. O trabalho mais recente, com resultados baseados em dados moleculares, considera Neoplecostomini (Lujan *et al*., 2015).

Neoplecostomus foi descrito por Eigenmann e Eigenmann (1888) como subgênero de *Plecostomus* (Gronovius 1854), mas foi considerado como gênero pelos mesmos autores em 1889. Os representantes de *Neoplecostomus* são animais de pequeno porte, encontrados em riachos com água rasa e clara, correnteza moderada a forte e fundo de pedras (Langeani, 1990). A diagnose do gênero baseia-se na presença de duas ou três séries de papilas bem desenvolvidas e proeminentes após cada série do dentário mais conspícuas que as demais presentes no restante do lábio e por possuir o ventre recoberto por um escudo, entre as nadadeiras peitorais e pélvicas, formado por pequenas placas e limitado por áreas nuas (Gosline, 1947; Langeani, 1990). O gênero inclui 18 espécies válidas e suas espécies são conhecidas por possuir morfologia conservada (Langeani, 1990; Roxo *et al*. 2012a). Desta forma, muitas espécies de *Neoplecostomus* são descritas com a integração de dados morfológicos e moleculares (Roxo *et al*., 2012a, 2014a, 2015).

DNA Barcoding para o reconhecimento de novas espécies

Nas últimas décadas, houve grandes avanços no desenvolvimento de novos marcadores moleculares, como fragmentos de DNA mitocondrial, que estão sendo combinados com métodos de metodologia molecular para reconhecer a biodiversidade (Bickford *et al*., 2006; Melo *et al*., 2011; Roxo *et al*., 2012a). A técnica de DNA Barcoding foi proposta por Hebert *et al*. (2003) com o objetivo primário de criar um sistema padrão, não ambíguo e rápido para identificar espécies usando sequências de DNA. Estudos usando esses métodos já foram publicados para diferentes grupos de organismos (Hebert *et al*., 2004; Ward *et al*., 2005; Clare *et al*., 2007; Kelly *et al*., 2007;

Hubert *et al*., 2008; Valdez-Moreno *et al*., 2009; Roxo *et al*., 2012a) e uma porcentagem significante desses estudos trazem uma grande resolução para identificação de espécimes.

Dessa forma, dentro dos objetivos do presente projeto está a aplicação do método de DNA Barcoding baseado no valor de 2% de divergência genética interespecífica proposto originalmente por Ward (2009), para delimitar as três espécies de *Neoplecostomus*, comparando também com as espécies descritas.

REFERÊNCIAS

Armbruster, J.W. (2004) Phylogenetic relationships of the sucker-mouth armored catfishes (Loricariidae) with particular emphasis on the Hypostominae and the Ancistrinae. *Zoological Journal of the Linnean Society*, 41, 1–80.

Bickford D., Lohman D.J., Sodhi N.S., Ng P.K.L., Meier R., Winker K., Ingram, K. K., & Das, I. (2006) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, 22 (3), 148–155.

Chiachio, M.C., Oliveira, C. & Montoya-Burgos, J.I. (2008) Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). *Molecular Phylogenetics and Evolution*, 49, 606–617.

Clare, E.L., Lim, B.K., Engstrom, M.D., Eger, J.L. & Hebert, P.D.N. (2007) DNA barcoding of Neotropical bats: species identification and discovery within Guyana. *Molecular Ecology Notes*, 7, 184–190.

Cuvier, G. & Valenciennes, (1840) *A. Histoire naturelle des poissons. Tome quinzième*. Suite du livre dix-septième. *Siluroïdes*, 15, 1–540.

Eigenmann, C.H. & Eigenmann, R.S. (1888) Preliminary notes on South American Nematognathi I. *Proceedings of the California Academy of Sciences*, 2, 119–172.

Eigenmann, C.H. & Eigenmann, R.S. (1889) Preliminary notes on South American Nematognathi II. *California Academy of Science*, 2, 25–59.

Fricke, R., Eschmeyer, W.N. & Fong, J.D. (2019) Catalog of fishes, Species by Family/ Subfamily. Availabe from: (http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp). (accessed 04 jan 2019).

Gosline, W. A. (1945) Catálogo dos Nematognatos de Água Doce da América do Sul e Central. *Boletim do Museu Nacional*, 33, 1–138.

Hebert, P.D.N., Cywinska A., Ball, S.L. & De Waard, J.R. (2003) Biological identifications through DNA barcodes. *Proceeding of the Royal Society B. Biological Science*, 270, 313–321.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences*, 101, 14812–14817.

Howes, G.J. (1983) The cranial muscles of loricarioid catfishes, their homologies and value as taxonomic characters (Teleostei: Siluroidei). *Bulletin of the British Museum Natural History*, 45(6), 309–345.

Hubert, N., Hanner, R.., Holm, E., Mandrak, N., Taylor, E., Burridge, M., Watkinson, D.A., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J. & Bernatchez, L. (2008) Identifying Canadian freshwater fishes through DNA barcodes. *PLoS One***,** 3(6): e2490.

Kelly, R.P., Sarkar, I.N., Eernisse, D.J. & Desalle, R. (2007) DNA barcoding using chitons (genus *Mopalia*). *Molecular Ecology Resources*, 7(2), 177–183.

Melo, B.F., Benine, R.C., Mariguela, T.C. & Oliveira C. (2011) A new species of *Tetragonopterus* Cuvier, 1816 (Characiformes: Characidae: Tetragonopterinae) from the rio Jari, Amapá, northern Brazil, *Neotropical Ichthyology*, 9(1), 49–56.

Langeani, F. (1990) Revisão do gênero *Neoplecostomus* Eigenmann & Eigenmann, 1888, com a descrição de quatro novas espécies do sudeste brasileiro (Ostariophysi, Silurformes, Loricariidae). *Comunicações do Museu de Ciências da PUCRS*, série Zoologia, 3, 3–31.

Lujan, N.K., Armbruster, J.W., Lovejoy, N.R. & López-Fernández, H. (2015) Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. *Molecular Phylogenetics and Evolution*, 82, 269–288.

Montoya-Burgos, J.I., Muller, S., Weber, C. & Pawlowski, J. (1998) Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. In: Malabarba*,* L.R., Reis, R.E., Vari, R.P., Lucena, Z.M & Lucena, C.A.S (Eds.), *Phylogeny and classification of neotropical fishes*. Edipucrs, pp. 363–374.

Nelson, J. S. (2006) *Fishes of the world*. 4ed. John Wiley and Sons, New York, 473 pp.

Pereira, E.H.L. & Reis, R.E. (2017) Morphology-based phylogeny of the suckermouth armored catfishes, with emphasis on the Neoplecostominae (Teleostei: Siluriformes: Loricariidae). *Zootaxa*, 4264 (1), 001–104.

Regan, C.T. (1904) A monograph of the fishes of the Family Loricariidae. *Transactions of The Zoological Society of London***,** London, 17, 191–350.

Reis, R.E., Kullander, O. & Ferraris-Jr, C. J. (2003) *Checklist of the freshwater fishes of South and Central America*. EDIPUCRS, Porto Alegre, 743pp.

Reis, R.E., Pereira, E.H.L. & Armbruster, J.W. (2006) Delturinae, a new catfish subfamily (Teleostei, Siluriformes), with revisions of *Delturus* and *Hemipsilichthys. Zoological Journal of the Linnean Society*, 147, 277–299.

Roxo, F.F., Oliveira, C. & Zawadzki, C.H. (2012a) Three new species of the *Neoplecostomus* (Teleostei: Siluriformes: Loricariidae) from the Upper Rio Paraná basin of southeastern Brazil. *Zootaxa*, 3233, 1–21.

Roxo, F.F., Silva, G.S.C., Zawadzki, C.H. & Oliveira, C. (2014a) *Neoplecostomus doceensis*: a new loricariid species (Teleostei, Siluriformes) from the Rio Doce basin and comments about its putative origin. *ZooKeys*, 440, 115–127.

Roxo, F.F., Ochoa, L.E., Costa-Silva, G.J. & Oliveira, C. (2015) Species delimitation in *Neoplecostomus* (Siluriformes: Loricariidae) using morphologic and genetic approaches. *DNA Barcodes*, 15, 110–117.

Schaefer, S.A. (1987) Osteology of *Hypostomus plecostomus* (Linnaeus) with a phylogenetic analysis of the loricariid subfamilies (Pisces: Siluroidei). *Contributions in Science*, 394, 1–31.

Silva, G.S.C., Roxo, F. F., Lujan, N.K., Tagliacollo, V.A., Zawadzki, C.H. & Oliveira, C. (2016) Transcontinental dispersal, ecological opportunity and origins of an adaptative radiation in the Neotropical catfish genus *Hypostomus* (Siluriformes: Loricariidae). *Molecular Ecology*, 25, 1511–1529.

Valdez-Moreno, M., Ivanova, N.V., Elías-Gutierez, M., Contreras-Balderas, S. & Hebert, P.D.N. (2009) Probing diversity in freshwater fishes from Mexico and Guatemala with DNA barcodes. *Journal of Fish Biology*, 74, 377–402.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Herbert, P.D.N. (2005) DNA barcoding Australia´s fish species. *Philosophical Transactions of the Royal Society B*, 360, 1847–1857.

Ward, R.D. (2009) DNA barcode divergence among species and genera of birds and fishes. *Molecular Ecology Resources*, 9(4), 1077–1085.

TWO NEW SPECIES OF *Neoplecostomus* (TELEOSTEI: SILURIFORMES) FROM THE UPPER PARANA SYSTEM BASED ON MORPHOLOGICAL AND MOLECULAR DATA

Artigo elaborado e formatado conforme as normas para publicação científica no periódico *Zootaxa*.

TWO NEW SPECIES OF *Neoplecostomus* (TELEOSTEI: SILURIFORMES) FROM THE UPPER PARANA SYSTEM BASED ON MORPHOLOGICAL AND MOLECULAR DATA

GABRIELA NARDI[1,5], CLÁUDIO HENRIQUE ZAWADZKI[2], FÁBIO FERNANDES ROXO[3] & CLÁUDIO OLIVEIRA[4]

[1]*Programa de Pós-Graduação em Biologia Comparada, Universidade Estadual de Maringá, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia), Av. Colombo 5790, G-80, Laboratório-Coleção de Peixes, Maringá, Paraná, Brazil*
[2]*Universidade Estadual de Maringá, Departamento de Biologia, Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura (Nupelia), Av. Colombo 5790, G-90, S. 18B, 87020-900, Maringá, Paraná state, Brazil*
[3]*Departamento de Zoologia, Instituto de Biociências, Universidade Estadual Paulista, R. Prof. Dr. Antonio C. W. Zanin, s/n Rubião Jr., 18618-689, Botucatu, São Paulo, state, Brazil*
[4]*Universidade Estadual Paulista, Departamento de Morfologia, Laboratório de Biologia e Genética de Peixes, R. Prof. Dr. Antonio C. W. Zanin, s/n Rubião Jr., 18618-689, Botucatu, São Paulo, state, Brazil*
[5]*Corresponding author. E-mail: gabriela.nardi@yahoo.com*

Abstract
In this study two new species of *Neoplecostomus* are described: *Neoplecostomus* sp. 1, from Piquiri basin, and *Neoplecostomus* sp. 2 from Ivaí basin, both from upper rio Paraná system, Paraná state. *Neoplecostomus* sp. 1 and *Neoplecostomus* sp. 2 can be distinguished from most congeners by having ill-developed adipose fin. Additionally, *Neoplecostomus* sp. 1 possess a distinct range of platelets of thoracic shield with the anterior edge straight, and the naked area around urogenital opening narrower than in other species due to the first ventral plates. *Neoplecostomus* sp. 2 can be also be distinguished by the round shape of head due to the pronounced meshthmoid, as by having greater orbital diameter/head length. Futhermore, we used DNA barcoding techniques of 2% threshold criteria to distinguish the new species from its congeners.

Key words: Neoplecostomini, Neotropical fish, freshwater fish, taxonomy, catfishes

INTRODUCTION

Hypoptopomatinae is a large subfamily of Loricariidae, with 243 valid species (Fricke *et al.* 2019) represented by small-sized catfishes (less than 110 mm SL), mostly restricted to southeastern Brazil and composed by Otothyrini, Neoplecostomini and Hypoptopomatini (Lujan *et al*. 2015). Neoplecostomini is currently represented by eight genera, *Euryochus* Pereira & Reis, 2017*, Hirtella* Pereira, Zanata, Cetra & Reis*,* 2014, *Isbrueckerichthys* Derijst, 1996, *Kronichthys* Mirando Ribeiro, 1908, *Neoplecostomus* Eigenmann & Eigenmann, 1888, *Pareiorhaphis* Miranda Ribeiro, 1918, *Pareiorhina* Gosline, 1947 (Pereira & Reis, 2017) and *Pseudotocinclus* Nichols, 1919.

Neoplecostomus is the second most representative genus for the tribe, with 18 valid species.

Neoplecostomus was redescribed by Langeani (1990), that recognized two valid species, *N. microps* (Steindachner, 1876) and *N. granosus* (Cuvier & Valenciennes, 1840), and described four new species: *N. paranensis* Langeani, 1990 from upper rio Paraná system, *N. ribeirensis* Langeani, 1990 from rio Ribeira de Iguape basin in São Paulo state, *N. espiritosantensis* Langeani, 1990 from streams of the eastern slopes of Serra do Mar in Espírito Santo State and *N. franciscoensis* Langeani, 1990 from rio São Francisco basin in Minas Gerais state. Later, Bizerril (1995) described *N. variipictus* from rio Paraíba do Sul basin. Posteriorly, several species were described for the upper rio Paraná system: *N. corumba* Zawadzki, Pavanelli & Langeani, 2008, *N. selenae* Zawadzki, Pavanelli & Langeani, 2008 and *N. yapo* Zawadzki, Pavanelli & Langeani, 2008, *N. bandeirante* Roxo, Oliveira & Zawadzki, 2012a*, N. botucatu* Roxo, Oliveira & Zawadzki, 2012a and *N. langeanii* Roxo, Oliveira & Zawadzki, 2012a, *N. jaguari* Andrade & Langeani 2014, *N. canastra* Roxo*,* Silva & Zawadzki, 2017, *N. watersi* Silva, Reia, Zawadzki & Roxo, 2019. Roxo *et al.* (2014a) described *N. doceensis* from the rio Doce basin and Cherobim *et al.* (2016) described *N. paraty* from rio Piquerê-Açu and surrounding basins in Rio de Janeiro State, totalizing 18 valid species.

The species of this group are small-sized fishes, found in small to medium-sized streams with clear water and substrates of loose stones and sand (Langeani, 1990). The species can be characterized by possessing: a ridge over the eyes, a swollen integument around the enlarged odontodes, a dermal flap along the dorsal portion of the unbranched pelvic-fin ray in males, a greater spinelet width influenced by the stage of development, as well as by sex (Langeani, 1990; Bizerril, 1995; Zawadzki *et al.*, 2008; Roxo *et al.,* 2012a). The members of this genus have as diagnostic characters the presence of: two or three series of well-developed papillae situated posteriorly to dentary teeth, platelets forming a shield between the insertion of pectoral and pelvic-fins, and pectoral-fin unbranched ray distinctly curved forming wide and conspicuous arch (Gosline, 1947; Langeani, 1990; Pereira & Reis, 2017).

Herein is used traditional morphological analyses for the genus *Neoplecostomus* and sequences of mithocondrial gene cytochrome c oxidase subunit I to genetically discriminate the species. These tools were used to separate, recognize and describe species of *Neoplecostomus* from upper rio Paraná system, specifically from rio Piquiri and rio Ivaí basins.

MATERIAL AND METHODS

Morphological analysis: Measurements and counts were taken from left side of specimens. Measurements were taken point to point with digital calipers to the nearest 0.1 mm following Zawadzki *et al*. (2008). Specimens from Ivaí and Piquiri basin smaller than 60.0 and 50.0 mm of SL, respectively, were not included in morphometric and meristics analysis, and are assigned as not measured. Plate counts followed Langeani (1990) modified by Zawadzki *et al*. (2008) and are present in Table 1. The analyzed samples of morphological data are deposited at (NUP) Coleção Ictiológica do Nupélia, Universidade Estadual de Maringá, municipality of Maringá, Paraná state; (LBP) Laboratório de Biologia Genética de Peixes, Universidade Estadual Paulista, municipality of Botucatu, São Paulo state; (MZUSP) Museu de Zoologia da Universidade de São Paulo, municipality of São Paulo, São Paulo state; (MCP) Museu de Ciência e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, municipality of Porto Alegre, Rio Grande do Sul state.

Genetic analysis: The analyzed samples for genetic data are listed in Table 2 and was used one sample of *Hisonotus nigricauda* as outgroup to root the tree and 58 sequences were taken from GenBank. The Voucher specimens are deposited at (NUP) Coleção Ictiológica do Nupélia, Universidade Estadual de Maringá, municipality of Maringá, Paraná state; (LBP) Laboratório de Biologia Genética de Peixes, Universidade Estadual Paulista, municipality of Botucatu, São Paulo state; and (DZSJRP) Coleção de Peixes do Departamento de Zoologia, municipality of São José do Rio Preto, São Paulo state.

Total DNA was extracted from ethanol preserved muscle, fin and liver samples with the protocol described by Aljanabi & Martinez (1997) or with the Wizard Genomic DNA Purification Kit (Promega). Partial sequences of the gene Cytochrome C oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR) using FishF1-5'-TCA ACC AAC CAC AAA GAC ATT GGC AC -3'and FishR1-5'-TAG ACT TCT GGG TGG CCA AAG AAT CA -3 as primers (Ward *et al.*, 2005). The PCR products identified on a 1% agarose gel. The PCR products were purified using ExoSap-IT following instructions of the manufacturer. The purified PCR products were used to make a sequencing PCR using the Kit "Big DyeTM Terminator v 3.1 Cycle Sequencing Ready Reaction" (Applied Biosystems). The amplified DNA was purified and loaded on an automatic sequencer.

DNA sequence and phylogenetic analyses: the consensus sequences were obtained with the program Geneious Pro 5.4.2 (Drummond *et al*., 2010). Posteriorly, all sequences for each gene were aligned using the algorithm MUSCLE (Edgar, 2004). The best nucleotide evolution model for COI gene, nucleotide variation, substitution patterns, and genetic distances were examined using MEGA 6.0 (Tamura *et al*., 2013). Phylogenetic analyses were performed using maximum likelihood (ML) analysis using RAxML HPC2 on XSEDE (Stamatakis, 2014) implemented on the CIPRES portal (Miller *et al.*, 2010) and performed using random starting trees with GTR+G model and keeping other parameters at default values. Bootstrap resampling (Felsenstein, 1985) was applied using 1000 pseudoreplicates to investigate the support of each node in the most likely topology.

Table 1. Species included in the present study. NUP = Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá; LBP = Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista; DZSJRP = Coleção de Peixes do Departamento de Zoologia, Universidade Estadual Paulista

	Collection No	Sample No	GenBank	Species	Location
1	LBP7384	34841	GQ214773	*Neoplecostomus ribeirensis*	Rio Água Doce, municipality of Tapiraí, São Paulo state, Ribeira do Iguape basin
2	LBP7384	34839	GQ214774	*N. ribeirensis*	Rio Água Doce, municipality of Tapiraí, São Paulo state, Ribeira do Iguape basin
3	LBP7384	34838	GQ214775	*N. ribeirensis*	Rio Água Doce, municipality of Tapiraí, São Paulo state, Ribeira do Iguape basin
4	LBP7384	34837	GQ214776	*N. ribeirensis*	Rio Água Doce, municipality of Tapiraí, São Paulo state, Ribeira do Iguape basin
5	NUP2528	9423	FJ434543	*N. corumba*	Córrego Gameleira, municipality of Corumbaíba, Goiás state, Rio Paranaíba basin
6	LBP7464	33413	GQ214778	*N. corumba*	Rio Taquarí, municipality of Carandaí, Minas Gerais state, Rio Paranaíba basin
7	LBP7464	33415	GQ214777	*N. corumba*	Rio Taquarí, municipality of Carandaí, Minas Gerais state, Rio Paranaíba basin
8	LBP7464	33411	GQ214780	*N. corumba*	Rio Taquarí, municipality of Carandaí, Minas Gerais state, Rio Paranaíba basin
9	LBP7464	33410	GQ214779	*N. corumba*	Rio Taquarí, municipality of Carandaí, Minas Gerais state, Rio Paranaíba basin
10	NUP3560	9700	GQ214782	*N. yapo*	Rio Forataleza, municipality of Tibagi, Paraná state, Rio Paranapanema basin
11	NUP3560	9701	FJ434529	*N. yapo*	Rio Forataleza, municipality of Tibagi, Paraná state, Rio Paranapanema basin
12	NUP3560	9699	GQ214783	*N. yapo*	Rio Forataleza, municipality of Tibagi, Paraná state, Rio Paranapanema basin
13	LBP5034	24681	GQ214781	*N. yapo*	Represa Três Pontes, municipality of Londrina, Paraná state, Rio Paranapanema basin
14	LBP5035	24683	FJ434533	*N. yapo*	Ribeirão Atlântico, municipality of Mandaguaçu, Paraná state, Rio Paranapanema basin
15	LBP7383	34843	FJ965506	*N. selenae*	Ribeirão das Batéias, municipality of Riacho Grande, São Paulo state, Rio Paranapanema basin
16	LBP7383	34844	GQ214791	*N. selenae*	Ribeirão das Batéias, municipality of Riacho Grande, São Paulo state, Rio Paranapanema basin
17	LBP7383	34846	GQ214789	*N. selenae*	Ribeirão das Batéias, municipality of Riacho Grande, São Paulo state, Rio Paranapanema basin
18	LBP7383	34842	GQ214792	*N. selenae*	Ribeirão das Batéias, municipality of Riacho Grande, São Paulo state, Rio Paranapanema basin
19	LBP7383	34845	GQ214790	*N. selenae*	Ribeirão das Batéias, municipality of Riacho Grande, São Paulo state, Rio Paranapanema basin
20	LBP7525	34834	GQ214788	*N. botucatu*	Ribeirão Água de Madalena, municipality of Botucatu, São Paulo state, Rio Paranapanema basin
21	LBP7525	34835	GQ214785	*N. botucatu*	Ribeirão Água de Madalena, municipality of Botucatu, São Paulo state, Rio Paranapanema basin
22	LBP7525	34836	GQ214787	*N. botucatu*	Ribeirão Água de Madalena, municipality of Botucatu, São Paulo state, Rio Paranapanema basin
23	LBP7525	34832	GQ214784	*N. botucatu*	Ribeirão Água de Madalena, municipality of Botucatu, São Paulo state, Rio Paranapanema basin
24	LBP7525	34833	GQ214786	*N. botucatu*	Ribeirão Água de Madalena, municipality of Botucatu, São Paulo state, Rio Paranapanema basin
25	LBP7466	33421	JN089810	*N. canastra*	Córrego Tamborete, municipality of Capitólio, Minas Gerais, Rio Grande basin

26	LBP7466	33425	KJ754544	*N. canastra*	Córrego Tamborete, municipality of Capitólio, Minas Gerais, Rio Grande basin
27	LBP2732	17441	FJ434532	*N. paranensis*	Córrego Mocoquinha, municipality of Cajuru, São Paulo state, Rio Grande basin
28	LBP2732	17446	GQ214803	*N. paranensis*	Córrego Mocoquinha, municipality of Cajuru, São Paulo state, Rio Grande basin
29	LBP2732	17448	GQ214802	*N. paranensis*	Córrego Mocoquinha, municipality of Cajuru, São Paulo state, Rio Grande basin
30	LBP2732	17444	FJ965505	*N. paranensis*	Córrego Mocoquinha, municipality of Cajuru, São Paulo state, Rio Grande basin
31	LBP6195	29290	GQ214797	*N. langeanii*	Rio Muzambinho, municipality of Muzambinho, Minas Gerais state, Rio Grande basin
32	LBP6195	29291	GQ214798	*N. langeanii*	Rio Muzambinho, municipality of Muzambinho, Minas Gerais state, Rio Grande basin
33	LBP5901	27990	GQ214800	*N. langeanii*	Rio Muzambinho, municipality of Muzambinho, Minas Gerais state, Rio Grande basin
34	LBP5901	27991	GQ214799	*N. langeanii*	Rio Muzambinho, municipality of Muzambinho, Minas Gerais state, Rio Grande basin
35	LBP6173	29260	GQ214801	*N. langeanii*	Córrego da Prata, municipality of Muzambinho, Minas Gerais state, Rio Grande basin
36	LBP2861	18613	GQ214793	*N. bandeirante*	Rio Paraitinga, municipality of Salenópolis, São Paulo state, Rio Tiête basin
37	LBP2861	18615	GQ214794	*N. bandeirante*	Rio Paraitinga, municipality of Salenópolis, São Paulo state, Rio Tiête basin
38	LBP2861	18612	GQ214795	*N. bandeirante*	Rio Paraitinga, municipality of Salenópolis, São Paulo state, Rio Tiête basin
39	LBP2861	18614	GQ214796	*N. bandeirante*	Rio Paraitinga, municipality of Salenópolis, São Paulo state, Rio Tiête basin
40	LBP1094	10232	GQ214807	*N. microps*	Ribeirão Fernandes, municipality of Sta. Bárbara do Tegúrio, Minas Gerais, Rio Paraíba do Sul basin
41	LBP6319	29383	GQ214805	*N. microps*	Ribeirão Água Santa, municipality of São José dos Barreiros, São Paulo state, Rio Paraíba do Sul basin
42	LBP6319	29385	GQ214813	*N. microps*	Ribeirão Água Santa, municipality of São José dos Barreiros, São Paulo state, Rio Paraíba do Sul basin
43	LBP6231	29382	GQ214806	*N. microps*	Ribeirão Água Santa, municipality of São José dos Barreiros, São Paulo state, Rio Paraíba do Sul basin
44	LBP645	7593	FJ434531	*N. microps*	Ribeirão Cajarana, municipality of Pindamonhangaba, São Paulo state, Paraíba do Sul basin
45	LBP2551	17104	GQ214808	*N. espiritosantensis*	Rio Jucu, municipality of Domingos Martins, Espírito Santos state, Oriental Coastal basin
46	LBP2551	17103	GQ214809	*N. espiritosantensis*	Rio Jucu, municipality of Domingos Martins, Espírito Santos state, Oriental Coastal basin
47	LBP2551	17102	GQ214810	*N. espiritosantensis*	Rio Jucu, municipality of Domingos Martins, Espírito Santos state, Oriental Coastal basin
48	LBP2551	15243	FJ434530	*N. espiritosantensis*	Rio Jucu, municipality of Domingos Martins, Espírito Santos state, Oriental Coastal basin
49	LBP6489	31533	GQ214804	*N. franciscoensis*	Rio das Velhas, municipality of São Bartolomeu, Minas Gerais state, Rio São Fr ancisco basin
50	LBP6486	31535	GQ214812	*N. franciscoensis*	Rio das Velhas, municipality of São Bartolomeu, Minas Gerais state, Rio São Francisco basin
51	LBP6493	31549	GQ214811	*N. franciscoensis*	Afluente Rio das Velhas, municipality of Ouro Preto, Minas Gerais state, Rio São Francisco basin
52	LBP6537	31681	FJ965504	*N. franciscoensis*	Afluente Rio das Velhas, municipality of Ouro Preto, Minas Gerais state, Rio Sã o Francisco basin
53	LBP7470	33442	KT362737	*N. jaguari*	Rio Jaguari, municipality of Camanducaia, Minas Gerais state, Rio Tietê basin
54	LBP7467	34712	KT362736	*N. jaguari*	Afluente Rio Jaguari, municipality of Camanducaia, Minas Gerais state, Rio Tietê basin
55	LBP7467	33427	KT362735	*N. jaguari*	Afluente Rio Jaguari, municipality of Camanducaia, Minas Gerais state, Rio Tietê basin
56	LBP7467	33426	KT362734	*N. jaguari*	Afluente Rio Jaguari, municipality of Camanducaia, Minas Gerais state, Rio Tietê basin
57	LBP1096	10268	JN089807	*N. doceensis*	Rio Chopotó, municipality of Desterro de Melo, Minas Gerais state, Rio Doce basin

58	NUP15730	89302	Not submitted	*Neoplescotomus* sp. 1	Rio Bonito, municipality of Goioxim, Paraná state, Rio Piquiri basin
59	NUP15767	89304	Not submitted	*Neoplescotomus* sp. 1	Rio Barreiro, municipality of Janiópolis, Paraná state, Rio Piquiri basin
60	LBP23387	95501	Not submitted	*Neoplecostomus* sp. 2	Afluente Rio Mourão, municipality of Campo Mourão, Paraná state, Ivaí basin
61	LBP23387	95502	Not submitted	*Neoplecostomus* sp. 2	Afluente Rio Mourão, municipality of Campo Mourão, Paraná state, Ivaí basin
62	LBP23387	95503	Not submitted	*Neoplecostomus* sp. 2	Afluente Rio Mourão, municipality of Campo Mourão, Paraná state, Ivaí basin
63	LBP23387	95504	Not submitted	*Neoplecostomus* sp. 2	Afluente Rio Mourão, municipality of Campo Mourão, Paraná state, Ivaí basin
64	LBP23387	95505	Not submitted	*Neoplecostomus* sp. 2	Afluente Rio Mourão, municipality of Campo Mourão, Paraná state, Ivaí basin

RESULTS

Neoplecostomus sp. n. 1, new species
Figure 1, Table 2

Neoplecostomus sp. – Delariva & Silva *et al*. (2013): Fish fauna of headwaters of Perobas Biological reserve; Cavalli *et al*. (2018): ichthyofauna of Piquiri river basin.

Holotype. MZUSP uncat., 71.5 mm SL, male, Brazil, Paraná state, municipality of Mamborê, Rio dos Macacos, Rio Piquiri basin, 24°15'12"S, 52°33'23"W, 28 November 2014, W. J. da Graça, W. M. Domingues, F. A. Teixeira and R. J. da Graça.

Paratypes. All from Brazil, Paraná state, rio Piquiri basin. NUP 14622, 1 male, 72.9 mm SL; 1 male, not measured; municipality of Tuneiras do Oeste, rio Concórdia, 23°15'32"S, 52°50'25"W, 17 March 2012, R. L. Delariva. NUP 17234, 3 males, 53.6–60.4 mm SL; 1 female, 54.9 mm SL; 1 male, not measured; municipality of Farol, córrego Água da Granada, 24°12'44"S, 52°47'50"W, 24 November 2014, C. H. Zawadzki. NUP 17222, 1 male, 65.4 mm SL; 1 female, 66.2 mm SL; municipality of Farol; 24°16'59"S, 52°41'26"W, 12 September 2014, C. H. Zawadzki. NUP 14631, 2 males, 69.5–69.8 mm SL; municipality of Tuneiras do Oeste, rio Concórdia; 23°52'55"S, 52°49'48"W, 16 March 2012, R. L. Delariva. NUP 17225, 3 females, 52.5–72.8 mm SL; 1 male, not measured; 1 female, not measured; municipality of Farol, rio Farol, 24°22'45"S, 52°40'54"W; 24 September 2014, C. H. Zawadzki. NUP 17230, 2 males, 64.0–72.8 mm SL; municipality of Farol; 24°22'53"S, 52°35'58"W; 24 September 2014, C. H. Zawadzki. NUP 15767, 1 female, 55.5 mm SL; municipality of Janiópolis, rio Barreiro, 24°12'44"S, 52°47'50"W; 29 January 2014, W. J. da Graça, W. M. Domingues, F. A. Teixeira and R. J. da Graça. NUP 15970, 1 male, not measured, collected with holotype.

Figure 1. *Neoplecostomus* sp. 1, MZUSP uncat., male, 71.5 mm SL, holotype from rio dos Macacos, municipality of Mamborê, Paraná state, Brazil.

Table 2. Morphometric data and counts of *Neoplecostomus* sp. 1 and *Neoplecostomus* sp. 2. SD = standard deviation, SL = standard length, CP = caudal peduncle, IO = interorbital width, OD = orbital diameter, PDS = predorsal, ds = dorsal fin, cd = caudal fin, ad = adipose fin, an = anal fin.

	Neoplecostomus sp. n. 2 (Ivaí) n = 32				*Neoplecostomus* sp. n. 1 (Piquiri) n= 15			
	Holotype	Range	Mean	SD	Holotype	Range	Mean	SD
Standard length	82.5	61.0–93.6	72.5	8.10	71.5	52.5–72.9	64.1	7.26
Percents of SL								
Predorsal length	41.0	38.6–43.1	41.2	1.05	41.5	39.0–41.5	40.3	0.67
Head length	30.5	27.3–32.0	30.0	1.05	29.8	27.2–30.0	29.1	0.65
Head width	26.2	23.6–27.3	25.5	0.85	24.2	21.6–25.4	26.7	0.78
Cleithral width	27.6	26.5–29.1	27.8	0.69	27.0	25.3–28.3	18.7	0.64
CP depth	7.3	6.1–8.0	7.0	0.51	7.6	6.6–8.1	9.3	0.23
Body depth	17.3	13.7–19.2	16.3	1.16	15.8	14.4–17.3	6.2	0.29
Preanal length	63.4	57.5–63.4	60.8	1.28	58.9	57.0–60.9	23.2	1.08
Percents of head length								
Head width	89.1	76.4–96.3	84.1	8.41	81.2	74.4–92.8	83.6	4.13
Head deph	53.5	48.2–56.5	52.7	2.28	50.7	36.4–59.1	51.8	5.75

Snout lengh	62.6	59.1–65.5	62.7	1.65	59.2	55.4–69.4	61.5	3.31
Orbital diameter	13.0	11.1–16.3	15.0	2.02	11.7	11.7–14.5	12.9	0.87
Interorbital width	34.8	30.8–35.5	33.5	1.25	37.1	34.0–39.4	36.5	1.43
Mandibular width	14.8	14.2–93.3	84.9	3.83	13.6	13.2–20.0	16.7	2.63
Other percents								
Snout length/OD	18.4	17.6–23.3	20.4	1.62	19.8	18.9–22.9	20.9	1.02
IO/OD	35.8	32.2–43.3	38.2	2.76	31.6	30.5–39.4	35.3	2.46
IO/mandibular width	56.8	41.1–59.3	49.3	6.15	36.7	35.6–56.3	45.8	6.98
PDS length/first ds length	49.7	24.9–51.3	47.0	4.47	49.5	44.7–51.7	48.6	2.01
CP lenght/CP depth	26.9	18.0–26.9	21.8	2.30	23.1	18.8–23.8	21.6	1.72
Pelvic-fin length/CP depth	30.6	25.0–34.0	29.5	2.07	34.8	31.0–36.6	33.5	1.62
Lower caudal spine/CP depth	33.5	24.3–33.9	29.2	2.35	30.5	25.5–32.6	29.8	1.83
Counts		Range	Mode			Range	Mode	
Lateral-line plates	29	27–30	29		28	27–30	29	
Predorsal plates	6	5–7	6		6	5–7	6	
Plates of dorsal-fin base	5	5–7	6		5	5–6	5	
Plates between ds and cd	19	15–19	17		17	**	17	
Plates between ad and cd	8	6–9	8		7	7–9	7	
Plates between an and cd	12	11–14	12		13	11–14	12	
Premaxillary teeth	19	16–29	16		12	12–19	17	
Dentary teeth	16	11–23	14		12	9–14	17	

Diagnosis. *Neoplecostomus* sp. n. 1 can be distinguished from all other congeners except *Neoplecostomus.* sp. 2, *N. botucatu* and *N. paranensis* by having a reduced adipose fin or azygous plates in the typical location of adipose fin (*vs*. total absence of an adipose fin or azygous plates in *N. watersi*; and well-developed adipose fin and always present in all other species). The new species can also be distinguished from all other congeners by having the naked area around urogenital opening narrower than in other species due to the first ventral plates (vs. wider naked area around urogenital opening), and by having the thoracic shield with its anterior edge straight and originating right at gill openings line (Fig. 2, 3) (*vs.* anterior edge thoracic shield with rounded shape and originating before gill openings).

Figure 2. Platelets and plates reach. *Neoplecostomus* sp. 1, NUP 17222, male, 65.4 mm, paratype. Black arrows showing the range of platelets (lateral view) and straight anterior edge of thoracic shield (ventral view). Red arrow showing first ventral plates covering part of abdominal region.

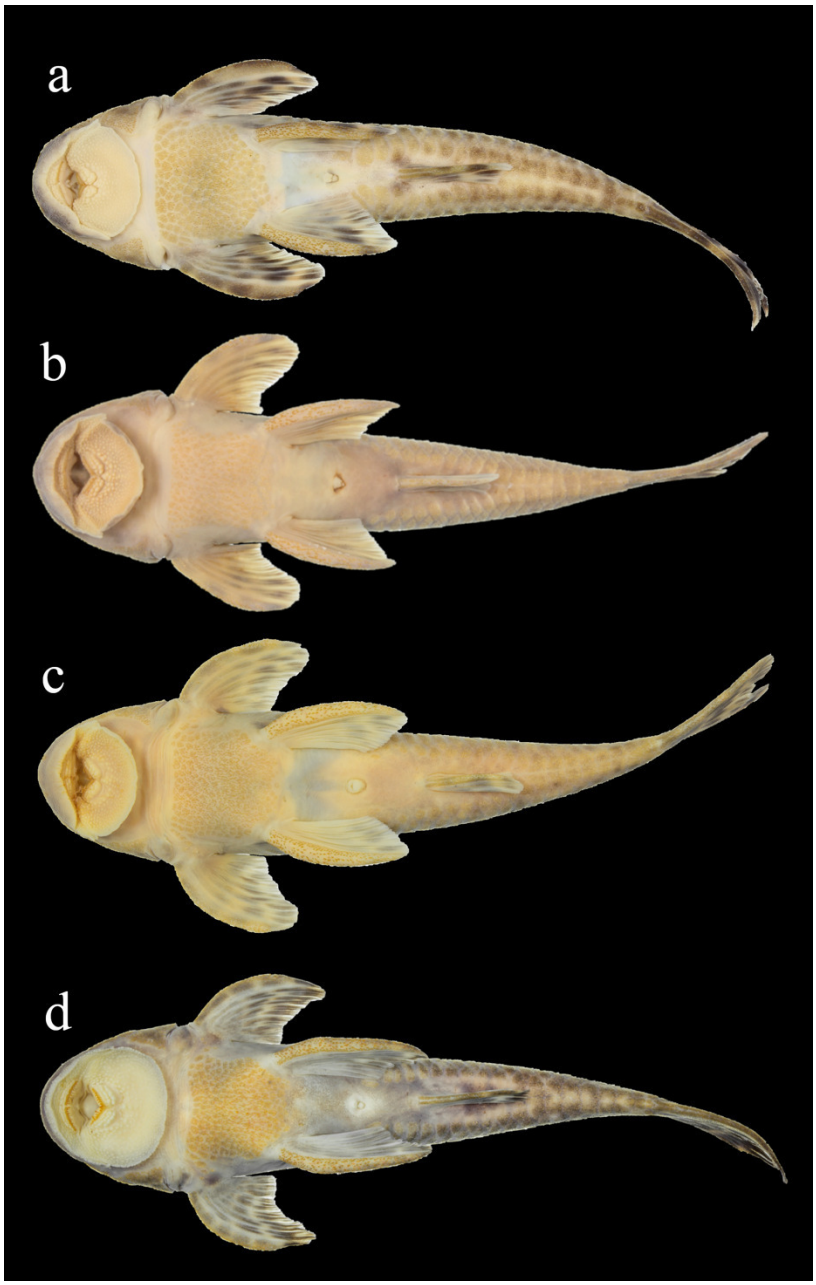

Figure 3. Ventral view comparing the range and reach of platelets of thoracic shield and abdominal region in *Neoplecostomus*. (a) *Neoplecostomus* sp. 1, paratype, NUP 14631, 70.1 mm SL; (b) *Neoplecostomus* sp. 2, paratype, NUP 10110, 68.6 mm SL; (c) *N. botucatu*, paratype, NUP 8015, 70.9 mm SL; (d) *N. watersi*, paratype, NUP 20525, 69.5 mm SL.

Description. Counts and measurements are presented in Table 2. Body elongate and depressed. Greatest width at cleithrum narrowing toward snout tip and posteriorly toward to caudal-fin. Dorsal profile of body gently convex, elevating from snout tip to dorsal-fin origin and descending to first caudal-fin procurrent ray. Greatest body depth at dorsal-fin origin. Trunk and caudal peduncle dorsally rounded in cross-section; body flattened ventrally to anal-fin origin, flattened to slightly rounded to caudal fin. Dorsal surface of body completely covered by dermal plates, excepting for a naked area around

dorsal-fin base. Snout tip naked. Ventral head surface naked, except by a plate ahead of gill openings. Abdomen with conspicuous, small dermal platelets between insertions of pectoral and pelvic fins, forming a thoracic shield surrounded by naked areas; wide thoracic shield passing pelvic-fin insertion and starting straight with gills openings. Area around urogenital opening partially covered by first ventral plates.

Head wide and depressed. Head and snout rounded in dorsal view. Interorbital space straight in frontal view. Median ridge rising from snout tip to area between nares. Snout convex in lateral profile. Odontodes from snout without distinct swollen skin. Eye moderately small (11.7–14.4% of HL), dorsolaterally placed. Lips well developed and rounded. Lower lip not reaching pectoral girdle and covered with papillae, wider anteriorly; two irregular and conspicuous rows of large and transversally flatted papillae, just posterior to dentary teeth. Maxillary barbel short, most coalesced with lower lip, generally with free tip. Teeth long, slender and biscuspid, bent inward, medial cusp longer than lateral. Dentary forming an angle of approximately 115°.

Dorsal fin i,7; its origin slightly posterior to vertical passing through pelvic-fin origin. Nuchal plate not covered by skin; dorsal-fin spinelet wider than dorsal-fin spine; dorsal-fin locking mechanism absent. Dorsal-fin posterior margin straight, reaching to or surpassing vertical through end of pelvic-fin when adpressed. Ill-developed adipose-fin. Pectoral fin i,6; spine depressed and curve inward, shorter than longest branched ray, its posterior margin slightly convex reaching first third pelvic-fin length when adpressed. Pelvic fin i,5; posterior margin nearly straight reaching to almost reaching anal-fin when adpressed. Pelvic-fin unbranched ray ventrally flattened, with dermal flap on its dorsal surface in males. Anal fin i,5; posterior margin nearly straight. Caudal fin i,14,i; furcated, lower lobe slightly longer than upper. Pectoral and pelvic-fin unbranched rays with odontodes on lateral and ventral portions. Anal-fin unbranched ray with odontodes only ventrally.

Color in alcohol. Dorsal surface ground color brownish with light or dark brown blotches. Head with straight yellowish line from snout tip to anterior nares. Light areas between and around eyes and posterior to opercle and preopercle. Dorsal coloration pattern usually retains the generic juvenile color pattern of five transversal darker bands, more subtle in adults specimens: first through the supraoccipital, second at origin of dorsal-fin, third at end of dorsal-fin, forth at adipose-fin and last at posterior portion of caudal peduncle. Body dorsally darker and ventrally lighter. All fins with irregular dark brown areas; sometimes forming inconspicuous transverse stripes.

Sexual dimorphism. Mature males with a dermal flap on the anterior dorsal surface of pelvic-fin unbranched ray and bearing a papilla in the urogenital opening.

Distribution. The specie is found in the tributaries of rio Piquiri, municipality of Mamborê, Farol, Tuneiras do Oeste, all from Paraná state, Brazil. (Fig. 4).

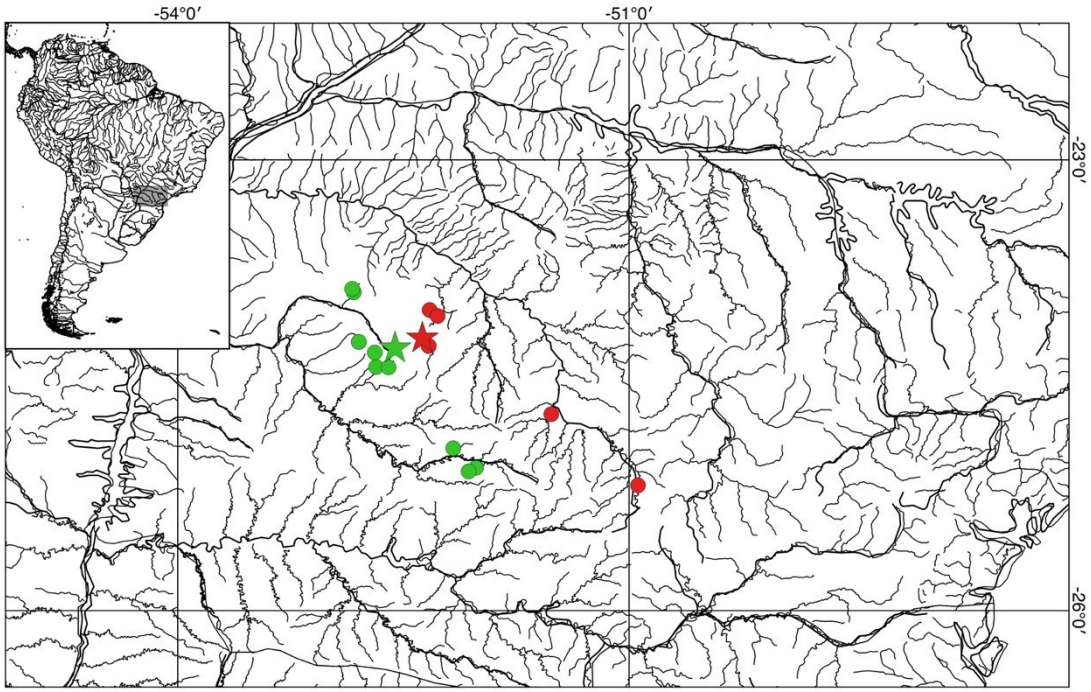

Figure 4. Distribution of the new species described in this study. Green markings represent the samples of *Neoplecostomus* sp. 1 from Piquiri basin and red markings *Neoplecostomus* sp. 2 from Ivaí basin. The stars represents the type locality and the circles the paratypes.

Neoplecostomus sp. n. 2, new species
Figure 5, Table 2

Neoplecostomus sp. – Frota *et al*. (2016): inventory of fish fauna of Ivaí river basin.

Holotype

MZUSP uncat., 82.5 mm SL, Paraná state, municipality of Campo Mourão, rio Mourão, upper Paraná basin, 24°11'14"S, 52°22'28"W, 01 May 2010, GERPEL staff (Grupo de Pesquisas em Recursos Pesqueiros e Limnologia).

Paratypes. All from Brazil, Parana state, rio Ivaí basin. NUP 10113, 2 males, 76.6–82.5 mm SL, 5 females, not measured, 7 sex not determined, not measured, collected with holotype. NUP 12063, 1 female, 64.8 mm SL, collected with holotype. NUP 10111, 3 males, 68.4–69.0 mm SL; 1 female, 69.8 mm SL, municipality of Campo Mourão, rio Mourão, 24°14'21"S, 52°19'57"W, 01 December 2009, GERPEL. NUP 10110, 6 males, 63.0–87.1 mm SL; 4 females, 72.8–77.6 mm SL; municipality of Campo Mourão, rio Mourão, 24°14'21"S, 52°19'57"W, 12 May 2010, GERPEL. NUP 10114, 1 male, 61.8 mm SL; 21 sex not determined, not measured, municipality of Campo Mourão, rio Mourão, 24°11'14"S, 52°22'28"W, 01 December 2009, GERPEL. NUP 6459, 1 male, 79.8 mm SL; municipality of Campo Mourão, rio Mourão, 24°00'00"S, 52°19'34"W, 15 December 2008, C. S. Pavanelli. NUP 15535, 4 males, 68.6–93.6 mm SL; 1 female, 61.0 mm SL; municipality of Prudentópolis, Rio dos Patos, 25°09'59"S, 50°56'29"W, 19 December 2013, C. H. Zawadzki et al. NUP 14976, 2 males, 68.9–71.9; municipality of Pitanga, rio Pitanga, 24°41'37"S, 51°31'03"W, 13 August 2013, I. Schneiberg & R. Rocha.

Figure 5. *Neoplecostomus* sp. 2, MZUSP uncat, male, 82.5 mm SL, holotype from rio Mourão, Municipality of Campo Mourão, Parana state, Brazil.

Diagnosis. *Neoplecostomus* sp. 2 is distinguished from all others congeners by having the anterior edge of mesethmoid rounded and higher, more evident in mature males (*vs*. straight or with mild curvature Fig. 6). *Neoplecostomus* sp. 2 differs from all others congeners except *Neoplecostomus* sp. 1, *N. botucatu* and *N. paranensis* due to the reduction in size of adipose-fin or presence of azygous plates in the typical position of adipose-fin (*vs*. total absence of an adipose fin or azygous plates in *N. watersi*; and well-developed adipose fin and always present in all other species); from *Neoplecostomus* sp. n. 1 differs by lacking ventral plates covering part of the abdominal region by and possessing the thoracic shield typically shaped, from *N. botucatu* is distinguished by having five dark bands on dorsum and lacking evident dark spots (vs. dark spots all over the body and fins) and by having greater orbital diameter 11.1–14.4% in head length, in

snout length 17.6–23.3%, and in interorbital width 32.2–43.3 (*vs.* 8.0–10%, 12.2–16.6% and 23.2–32.0%) and smaller head depth in head length 48.2–56.5% (*vs.* 55.6–63.9%); *Neoplecostomus* sp. 2 differs from *N. paranensis* by having greater orbital diameter 11.1–14.4% in head length, 17.6–23.3% in snout length and, 32.2–43.3% in interorbital length (vs. 7.9–12.0%, 12.4–16.7% and, 27.6–32.4% respectively), mandibular width 14.2–19.6% in head length (vs. 8.4–12.4%).

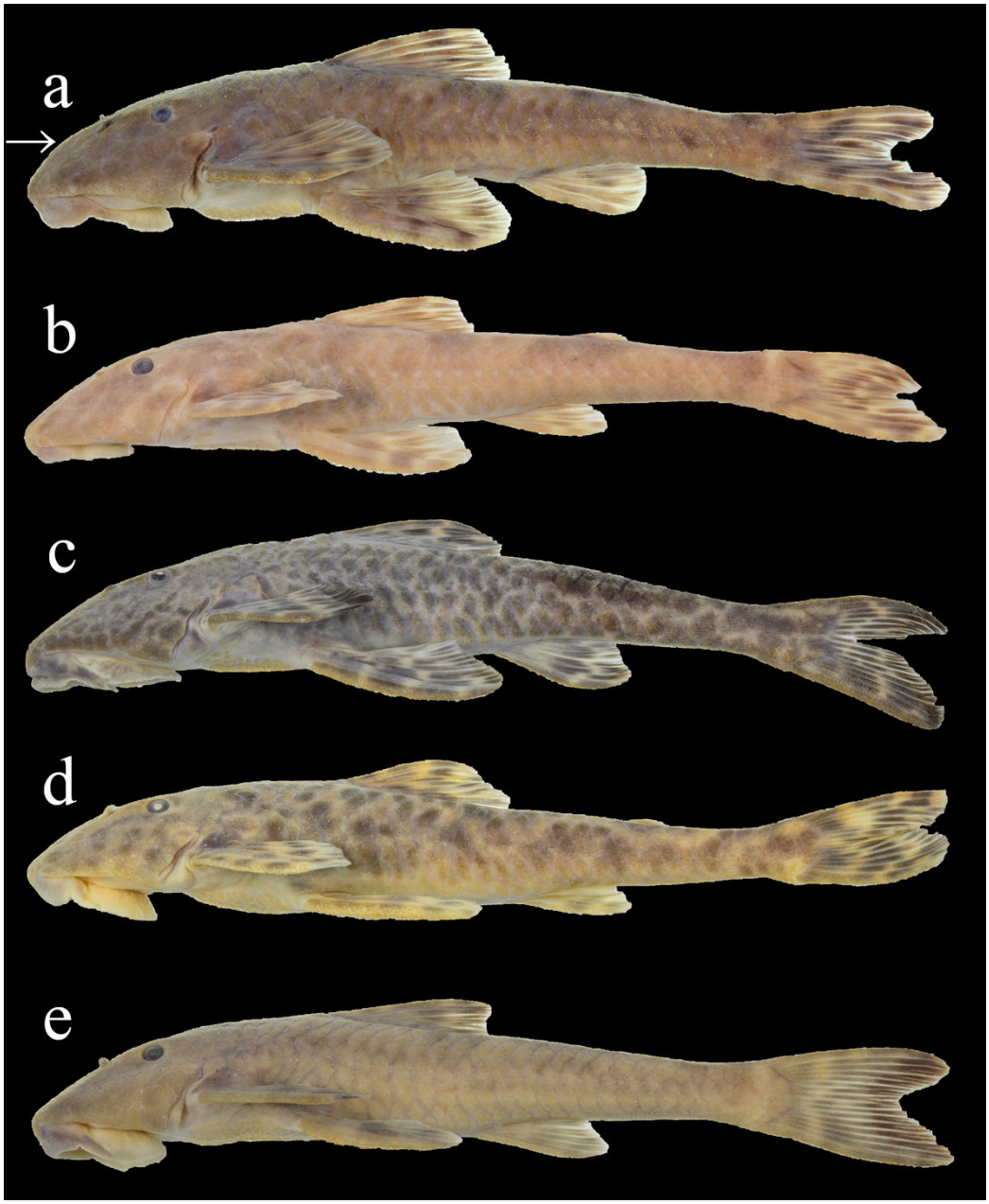

Figure 6. Lateral view highlighting the rounded shape of head in *Neoplecostomus* sp. 2 compared to other species. (a) *Neoplecostomus* sp. 2, paratype, NUP 10113, 70.0 mm SL; (b) *N. paranensis*, holotype, MZUSP 38572, 71.4 mm SL; (c) *N. watersi*, paratype, NUP 20525, 69.5 mm SL; (d) *N. botucatu*, paratype, NUP 8015, 70.9 mm SL; (e) *Neoplecostomus* sp. 1, MZUSP uncat., holotype, 71.5 mm SL.

Description. Counts and measurements are presented in Table 1. Body elongate and depressed. Greatest width at cleithrum narrowing toward caudal-fin. Dorsal profile of body gently convex, elevating from snout tip to dorsal-fin origin and descending to first caudal-fin procurrent spine. Greatest body depth at dorsal-fin origin. Trunk and caudal peduncle dorsally rounded in cross-section; body flattened ventrally to anal-fin origin, flattened to slightly rounded to caudal fin. Dorsal body surface covered by dermal plates, excepting for a naked area around dorsal-fin base. Snout tip naked. Ventral surface of head naked except by platelets bearing odontodes in front of gill openings.

Abdomen with conspicuous, small dermal platelets between insertions of pectoral and pelvic fins, forming thoracic shield surrounded by naked areas, with some few platelets toward pectoral fin insertion. Head wide and depressed. Head and snout rounded in dorsal view. Interorbital space straight to slightly concave in frontal view. Pronounced mesethmoid forming a Great ridge rising from snout tip to area between nares. A ridge from nares to superior margin of orbit. Odontodes from snout without distinct swollen skin. Eye relatively small (11.1–14.4% of HL), dorsolaterally placed. Lips well developed and rounded. Lower lip not reaching pectoral girdle and covered with papillae; papillae wider proximaly; two irregular and conspicuous rows of large and transversally flattned papillae, just posterior to dentary teeth. Maxillary barbell short, most coalesced with lower lip, usually its tip not free from lower lip. Teeth long, slender, bent inward and biscuspid, medial cusp longer than lateral. Dentary forming an angle of approximately 120°.

Dorsal fin i,7; origin slightly posterior to pelvic-fin origin. Nuchal plate not covered by skin; dorsal-fin spinelet wider than dorsal-fin spine; dorsal-fin locking mechanism not functional. Dorsal fin posterior margin straight, reaching to or surpassing vertical through end of pelvic-fin when adpressed. Ill-developed adipose fin. Pectoral fin i,6; spine depressed and curve inward (more pronounced in large specimens), spine shorter than longest branched ray. Pectoral fin posterior margin slightly convex; reaching first third of pelvic-fin length when adpressed. Pelvic fin i,5; flexible; its posterior margin nearly straight; reaching or almost reaching anal fin when adpressed. Pelvic-fin unbranched ray ventrally flattened, with dermal flap on its dorsal surface in males. Anal-fin i,5; its posterior margin nearly straight. Caudal-fin i,14,i; furcated, lower lobe slightly longer than upper. Pectoral spine and pelvic-fin unbranched rays with odontodes on lateral and ventral portions. Anal-fin unbranched ray with odontodes only ventrally.

Color in alcohol. Dorsal surface ground color brownish with light or dark brown blotches. Small light areas on interobital region and around opercle and preopercle. Dorsal coloration pattern retains the generic juvenile color pattern of five transversal darker bands: the first through the supraoccipital, the second at origin of dorsal-fin, the third at end of dorsal-fin, the forth at adipose-fin and the last at posterior portion of caudal peduncle. Body dorsally dark and ventrally lighter. Pectoral-fins anteriorly darker and posteriorly lighter. All fins with irregular dark brown areas; sometimes forming inconspicuous transverse stripes.

Sexual dimorphism. Mature males with a dermal flap on the anterior dorsal surface of pelvic-fin unbranched ray and bearing a papilla in the urogenital opening.

Distribution The specie is found in the tributaries of rio Ivaí, municipality of Campo Mourão, Prudentópolis and Pitanga, all from Paraná state, Brazil (Fig. 4).

Genetic Analysis

It was sequenced COI gene from seven samples of the two new species of *Neoplecostomus* described herein. It was used 58 sequences available on GenBank representing 15 valid species of *Neoplecosotmus*. The final matrix has 559 pb of COI obtained from a total of 65 specimens. The nucleotide frequencies are A= 24.0%, T/U= 25.6%, C= 32.1% and G= 18.3%. The best model of nucleotide evolution for COI gene evaluated was TN93+G.

All the the specimens of *Neoplecostomus* formed monophyletic clusters, with different levels of genetic divergence between those clusters (Fig. 5). Genetic distances among *Neoplecostomus* species are shown in Table 3. The rate of genetic variation ranges from 1.0% between *N. selenae* and *N. botucatu*, to 12.4% among *N. ribeirensis* and *N. franciscoensis*. In the final tree, it was found two clusters corresponding to the new species and 16 corresponding to previously described species.

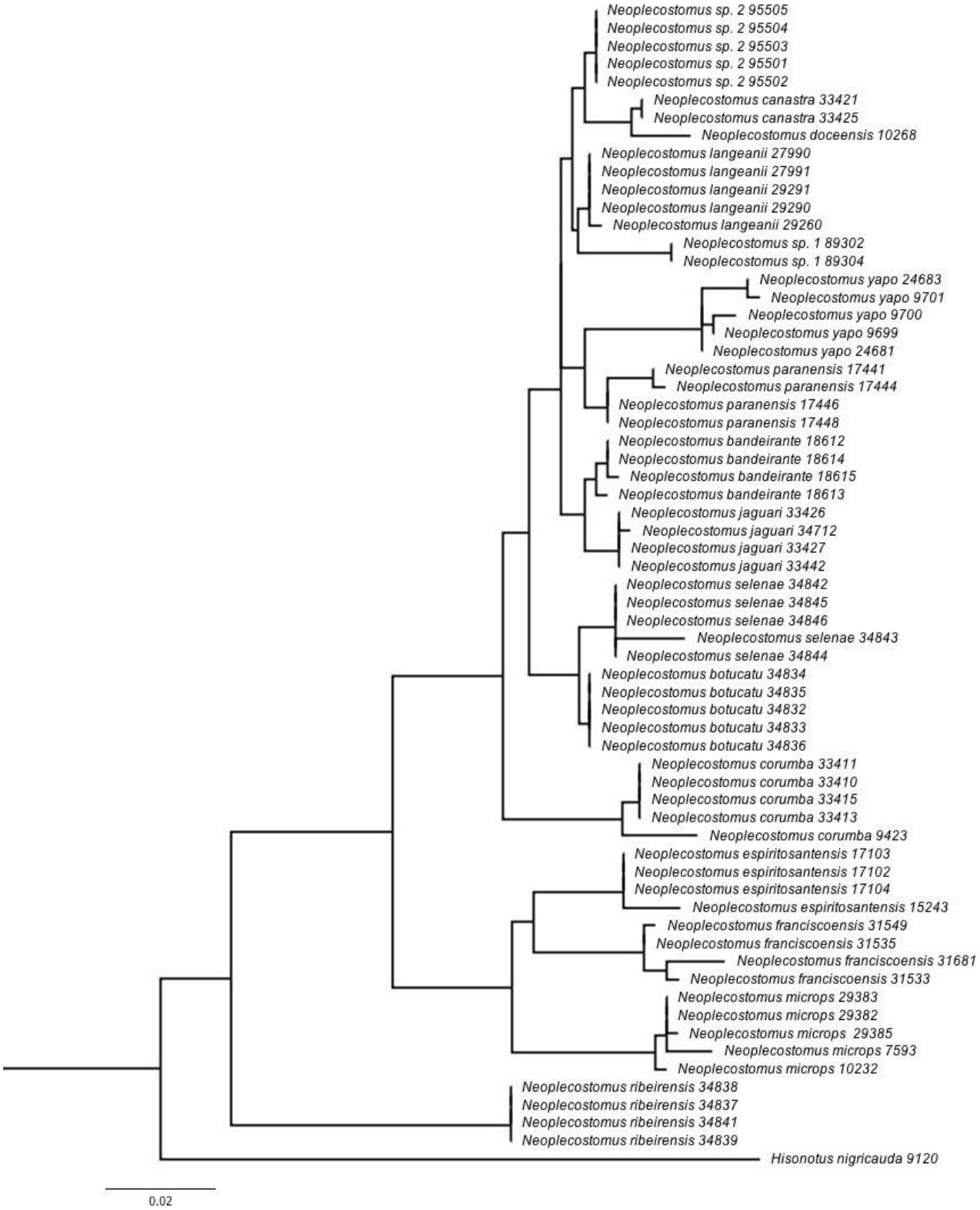

Figure 7. Phylogenetic tree of species of *Neoplecostomus* constructed using ML, based on the COI (559 pb).

Table 3. Genetic distance between *Neoplecostomus* species and specimens of the same species (main diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 - *N. ribeirensis*	0,00															
2 - *N. corumba*	0,12	0,01														
3 - *N. yapo*	0,11	0,06	0,01													
4 - *N. selenae*	0,11	0,05	0,04	0,01												
5 - *N. botucatu*	0,10	0,04	0,04	0,01	0,00											
6 - *N. doceensis*	0,11	0,06	0,05	0,04	0,03	–										
7 - N. bandeirante	0,10	0,04	0,04	0,03	0,03	0,03	0,00									
8 - *N.* sp.1	0,11	0,05	0,04	0,04	0,04	0,04	0,03	0,00								
9 - *N. jaguari*	0,11	0,04	0,04	0,04	0,03	0,03	0,01	0,03	0,00							
10 - *N. canastra*	0,10	0,05	0,05	0,03	0,03	0,01	0,02	0,03	0,02	0,00						
11 - *N.* sp. 2	0,10	0,04	0,04	0,03	0,02	0,02	0,02	0,02	0,02	0,01	0,00					
12 - *N. langeanii*	0,10	0,04	0,04	0,03	0,02	0,02	0,01	0,02	0,02	0,01	0,01	0,00				
13 - *N. paranensis*	0,11	0,04	0,03	0,04	0,03	0,04	0,02	0,03	0,02	0,03	0,02	0,02	0,01			
14 - *N. microps*	0,12	0,09	0,10	0,10	0,09	0,10	0,09	0,09	0,09	0,09	0,09	0,09	0,09	0,01		
15 - *N. espiritosantensis*	0,12	0,07	0,08	0,08	0,07	0,09	0,07	0,08	0,08	0,08	0,07	0,07	0,06	0,05	0,01	
16 - *N. franciscoensis*	0,13	0,09	0,08	0,09	0,08	0,10	0,08	0,09	0,08	0,09	0,09	0,08	0,08	0,06	0,05	0,01

DISCUSSION

Studies using DNA sequence datasets have been used for many groups of animals as a good way to report resolution in the identification of the biodiversity (Hebert *et al.*, 2004; Clare *et al.*, 2007; García-Robledo *et al.*, 2015; Melo *et al.*, 2016). Due to the high degree of morphological similarity between representatives of *Neoplecostomus*, the combination with molecular markers to identify and characterize the species have been very helpful (e.g., Zawadzki *et al*., 2004; Roxo *et al.* 2012a, 2015, 2017). Therefore, our results have shown that all populations of *Neoplecostomus* analyzed formed monophyletic clusters and the molecular analysis strongly support that *Neoplecostomus* sp. 1 e *Neoplecostomus* sp. 2 are unique evolutionary lineages (Fig. 7).

Neoplecostomus sp. 1 and *Neoplecostomus* sp. 2 possess the typical diagnostic characters of the genus: as two series of papillae just posterior to the dentary teeth, platelets forming a shield between the insertion of pectoral and pelvic-fins; and pectoral-fin unbranched ray distinctly curved (Gosline, 1947; Langeani, 1990; Pereira & Reis, 2017). The main characters used to distinguish the new species from its congeners are the reduction in size of adipose-fin in both species; the format and range of thoracic shield, as first initial plates of ventral series in *Neoplecostomus* sp. 1 (Fig. 2,3); the rounded shape of head in lateral view provided by the pronounced mesethmoid in *Neoplecostomus* sp. 2 (Fig. 6). A poor developed adipose fin is a character already used to distinguish species of *Neoplecostomus* (e.g. *N. paranensis* and *N. botucatu*), and the most recent study with this genus describes *N. watersi*, the first species that completely lacks an adipose fin (Silva *et al*. 2019).

Low genetic divergence between *Neoplecostomus* species was reported in previous studies, between *N. botucatu* and *N. selenae* and also between *N. canastra* and *N. langeanii* (Roxo et al., 2012a; 2017, respectively) recognized as the same species in molecular analysis, but distinct in alpha taxonomy, possibly related to the recent diversification of species of the genus (Roxo *et al*., 2012a;2012b; 2014b; 2015; 2017). The distribution of *Neoplecostomus* in both basins (Piquiri and Ivaí) suggests shared patterns of species endemism and historical drainage connections between them as reported by Cavalli *et al*. (2018). Besides, the species of *Neoplecostomus* are small-sized catfishes that can attach to rocks with its spines and resist to water flow that also helps with a low migration range. Therefore, reduction in gene flow and endogamic genetic events could favor fast speciation processes (Zawadzki *et al*. 2008), these features plus the recent speciation of the genus can promote high endemism. For this reason, the species

usually form single clusters per locality (Fig. 7) which agrees with Zawadzki *et al*. (2004), that populations of *Neoplecostomus* have a conserved morphology but are isolated, with restrict gene flow and geographic distribution as observed in *N. bandeirante, N. langeanii, N. botucatu and N. canastra* known only for their type-locality.

The recognition of endemic zones is of great importance, once that narrow endemic or restricted-range species indicate sites for which there are few spatial options of conservation (Eken *et al*., 2004). The rio Piquiri and rio Ivai basin harbor great number of species, which some of them are rare, endemic and even unknown by science (Frota *et al*., 2016; Cavalli *et al*., 2018). The identification of new species with low genetic divergence and conserved morphology, as observed in *Neoplecostomus*, is important for the conservation of these species, especially in species that have small geographic distribution, therefore more susceptible to extinction (Zawadzki *et al.*, 2008; Roxo *et al*., 2012a). Futhermore, we can highlight the efficiency of both tools (morphological and molecular) in distinguish *Neoplecostomus* species and the combination of them is a better way to understand not just possible variations but the evolutionary process involving the taxa and their locations.

COMPARATIVE MATERIALS

Neoplecostomus bandeirante Roxo, Oliveira & Zawadzki, 2012: holotype, MZUSP 110363, 109.9 mm SL, rio Paraitinguinha, rio Tietê basin; paratypes, MZUSP 087141, 6, 28.2–35.6 mm SL, rio Claro, rio Tietê, LBP 2861, 4, 55,6–93.9 mm SL.

Neoplecostomus botucatu Roxo, Oliveira & Zawadzki, 2012: holotype, MZUSP 110364, 98.6 mm SL, córrego Águas de Madalena, rio Paranapanema basin; paratypes, MZUSP 110362, 3, 75.4–88.0 mm SL, rio Pardo, rio Paranapanema basin, LBP 8065, 12, 67.0–87.0 mm SL, córrego Águas de Madalena, NUP 8016, 1, 69.8 mm SL, córego Águas de Madalena.

Neoplecostomus canastra Roxo, Silva, Zawadzki & Oliveira, 2017: holotype MZUSP 121504, 82.5 mm SL, córrego Tamborete, rio Grande basin.

Neoplecostomus corumba Zawadzki, Pavanelli & Langeni, 2008: paratypes, MZUSP 86208, 9, 44.0–75.4 mm SL, córredo Gameleira, rio Paranaíba basin, NUP 2528, 5, 52.0–67.0 mm SL, córrego Gameleira, rio Paranaíba basin.

Neoplecostomus doceensis Roxo, Silva, Zawadzki & Oliveira, 2014: holotype, MZUSP 115486, 101.1 mm SL, córrego Bananeiras, rio Doce basin; paratypes, MZUSP

110931, 2, 63.1–80.0 mm SL, rio Galaxo do Sul, rio Doce basin, MZUSP 94542, 9, 36.3–53.8 mm SL, rio Xopotó, rio Doce basin.

Neoplecostomus espiritosantensis Langeani, 1990: holotype MZUSP 038573, 101.1 mm SL, rio Jucu, Coastal Drainage;

Neoplecostomu franciscoensis Langeani, 1990: holotype MZUSP 38577, 68.6 mm SL tributary of córrego Mutuca, rio São Francisco basin; MZUSP 37163, 2, 37.9–42.4 mm SL, tributary of rio Paraopeba, rio São Francisco basin; MZUSP 037171, 4 40.1–42.0 mm SL.

Neoplecostomus jaguari Andrade & Langeani, 2014: MZUSP 10616, 1, 93.5 mm SL, córrego sem nome, rio Tietê basin.

Neoplecostomus langeanii Roxo, Oliveira & Zawadzki, 2012: holotype MZUSP 110365, 77.2 mm SL, tributary of rio São Domingos, rio Grande basin; paratypes MZUSP 110361, 1, 84.0 mm SL, rio Muzambinho, rio Grande basin; MZUSP 110360, 6, 52.1–71.4 mm SL rio São Domingos, rio Grande basin; LBP, 4, 58.5–74.0 mm SL, rio Muzambinho, rio Grande basin.

Neoplecostomus microps (Steindachner, 1877): MZUSP 11088, 80.7 mm SL, rio Bananal, Paraíba do Sul basin; MZUSP 109846, 84.8 mm SL, rio Bananal, Paraíba do Sul basin. LBP 6296, 10, 54.6–81.1 mm SL, rio Macaquinho, rio Paraíba do Sul basin.

Neoplecostomus paranensis Langeani, 1990: holotype, MZUSP 38572, 71.4 mm SL, rio Cubatão, rio Grande basin; paratypes, MZUSP 36625, 1, 55.7 mm SL, rio São Bartolomeu, rio Grande basin; MZUSP 38822, 92.3 mm SL, rio Cubatão, rio Grande basin, MZUSP 38823, 88.2 mm SL, rio Cubatão, rio Grande basin, MZUSP, 38824, 67.9 mm SL, rio Cubatão, rio Grande basin.

Neoplecostomus ribeirensis Langeani, 1990: holotype, MZUSP 038576, 86.9 mm SL, tributary of rio Bananal, rio Ribeira de Iguape basin.

Neoplecostomus selenae Zawadzki, Pavanelli & Langeani, 2008: holotype, MZUSP 51889, 101.7 mm SL, Ribeirão das Batatéias, rio Paranapanema basin; paratypes MZUSP 52589, 4, 41.6–64.2 mm SL Ribeirão das Batatéias, rio Paranapanema basin; MZUSP 51873, 55.7 mm SL, Ribeirão das Batatéias, rio Paranapanema basin.

Neoplecostomus yapo Zawadzki, Pavanelli & Langeani, 2008: paratype MZUSP 63.3–104.3 mm SL, tributary of rio Yapó, rio Paranapanema basin; NUP 2609, 15, 48.0–110.0 mm SL, riacho Fortaleza, rio Paranapanema basin.

REFERENCES

Albert, J.S. & Reis, R.E. (2011) *Historical biogeography of Neotropical freshwater fishes*. University of California Press, Barkele, Los Angeles, London.

Aljanabi, S. M. & Martinez, I. (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25 (22), 4692–4693.

Andrade, B.N. & Langeani, F.A. (2014) New species of *Neoplecostomus* Eigenmann & Eigenmann, 1888 (Siluriformes: Loricariidae: Neoplecostominae) from the upper rio Paraná basin. *Neotropical Ichthyology*, 12, 675–681.

Bizerril, C.R.S.F. (1995) Description of a new species of *Neoplecostomus* (Loricaridae, Neoplecostominae), with a synopsis of the taxonomical composition of the Loricariidae in eastern Brasil. *Arquivos de Biologia e Tecnologia*, 38, 693–704.

Cavalli, D., Frota, A., Lira A.D., Gubiani, E.A., Margarido, V.P. & da Graça, W.J. (2018) Update on the ichthyofauna of the Piquiri River basin, Paraná, Brazil: a conservation priority area. *Biota Neotropica*, 18(2): e20170350.

Cherobim, A.M., Lazzarotto, H. & Langeani, F. (2016) A new species of the catfish *Neoplecostomus* (Loricariidae: Neoplecostominae) from a coastal drainage in southeastern Brazil. *Neotropical Ichthyology*, 14(4): e160015.

Clare E.L., Lim B.K., Engstrom M.D., Eger J.L., Hebert P.D.N. (2007) DNA barcoding of Neotropical bats: Species identification and discovery within Guyana. *Molecular Ecology Notes*, 7, 184–190.

Cuvier, G. & Valenciennes, (1840) A. Histoire naturelle des poissons. Tome quinzième. Suite du livre dix-septième. *Siluroïdes*, 15, 1–540.

Deprá, G.C., Graça, W.J., Pavanelli, C.S., Avelino, G. S. & Oliveira, C. (2018) Molecular phylogeny of Planaltina Böhlke (Characidae: Stevardiinae) and comments on the definition and geographic distribution of the genus, with description of a new species. *Plos One*, 13(5), 1–26.

Delariva, R.L. & Silva, J.C. (2013) Fish fauna of headwaters streams of Perobas Biological Reserve, a conservation unit in the Atlantic Forest of the Northwestern Paraná State, Brazil. *Checklist*, 9(3), 549–554.

Derijst, E. (1996) Note on the type species of the mailed catfish genus *Pareiorhaphis* Miranda-Ribeiro, 1918 (Pisces: Siluriformes: Loricariidae), with the introduction of the *Isbrueckerichthys* nom. Nov. *Aquarium Wereld*, 3, 62–64.

Drummond, A., Sshton, B., Buxton, S., Cheung, M., Cooper, A., et al., (2010) *Geneious*. Book Geneious.

Edgar, R.C. (2004) Muscle: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5, 1–19.

Eigenmann, C.H. & Eigenmann, R.S. (1888) Preliminary notes on South American Nematognathi I. *Proceedings of the California Academy of Sciences*, 2, 119–172.

Eken G., Bennun L, Brooks T.M, Darwall W., Fishpool L.D.C., et al. (2004) Key biodiversity areas as site conservation targets. *BioScience*, 54, 1110–1118.

Felsenstein, J. (1985) Phylogenies and the comparative method. *The American Naturalist*, 125 (1), 1–15.

Frota, A., Deprá, G.C., Petenucci, L.M., da Graça, W.J. (2016) Inventor of the fish fauna from Ivaí River basin, Paraná State, Brazil. *Biota Neotropica*, 16(3): e20150151.

Fricke, R., Eschmeyer, W.N. & Fong, J.D. (2019) Catalog of fishes, Species by Family/ Subfamily. Availabe from: (http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp). (accessed 04 jan 2019).

García-Robledo C., Staines C.L., Kress W.J. (2015) A new species of bromeliad-feeding Cephaloleia Chevrolat (Coleoptera, Chrysomelidae, Cassidinae) from Costa Rica: evidence from DNA barcodes, larval and adult morphology and insect diets. *ZooKeys,* 477, 143–155.

Gosline, W. A. (1945) Catálogo dos Nematognatos de Água Doce da América do Sul e Central. *Boletim do Museu Nacional*, 33, 1-138.

Gosline, W.A. (1947) Contributions to the classification of the loricariid catfishes. *Arquivos do Museu Nacional do Rio de Janeiro*, 41,79–134.

Hausdorf, B. (2002) Units in biogeography. *Systematic Zoology*, 51, 648–652.

Hebert P.D.N., Stoeckle M.Y., Zemlak T.S., Francis C.M. (2004) Identification of birds through DNA barcodes. *PLoS Biology*, 2, 1657–1663.

Langeani, F. (1990) Revisão do gênero *Neoplecostomus* Eigenmann & Eigenmann, 1888, com a descrição de quatro novas espécies do sudeste brasileiro (Ostariophysi, Silurformes, Loricariidae). *Comunicações do Museu de Ciências da PUCRS*, série Zoologia, Porto Alegre, 3, 3–31.

Langeani, F., Castro, R.M.C., Oyakawa, O.T., Shibatta, O.A., Pavanelli, C.S. & Casatti, L. (2007) Diversidade da ictiofauna do alto rio Paraná: composição atual e perspectivas futuras. *Biota Neotropica*, 7, 181–197.

Lujan, N.K., Armbruster, J.W., Lovejoy, N.R. & López-Fernández, H. (2015) Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. *Molecular Phylogenetics and Evolution*, Toronto, 82, 269–288.

Melo, B.F., Ochoa, L.E., Vari, R.P., Oliveira, C. (2016) Cryptic species in the Neotropical fish genus *Curimatopsis* (Teleostei, Characiformes). *Zoologica Scripta*, 45, 650–658.

Miller, M; Pfeiffer, W.; Shwartz, T. (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE*), 14 Nov., New Orleans, LA, 1–8 pp.

Miranda Ribeiro, A. (1908) On fishes from Iporanga River. S. Paulo-Brasil. *Arkiv för Zoologi*, 4, 1–5.

Miranda Ribeiro, A. (1918) *Hemipsilichthys*, Eingenmann & Eingenmann e gêneros aliados. *Revista da Sociedade Brasileira de Ciências*, 2, 101–107.

Morrone, J.J. (1994) On the identification of areas of endemism. *Systematic Biology*, 43(3), 438–441.

Nichols, J.T. (1919) Um novo gênero de cascudos da família Loricariidae. *Revista do Museu Paulista*, 11, 533–535.

Pereira, E.H.L. & Reis, R.E. (2017) Morphology-based phylogeny of the suckermouth armored catfishes, with emphasis on the Neoplecostominae (Teleostei: Siluriformes: Loricariidae). *Zootaxa*, 4264 (1), 1–104.

Pereira, E.H.L., Zanata, A., Cetra, M. & Reis, R.E. (2014) A remarkable sexually dimorphic new genus and species of Neoplecostominae catfish (Siluriformes, Loricariidae) from coastal drainage of esastern Brazil. *Copeia*, 673, 673–681.

Ribeiro, A.C., Lima, F.C.T. & Menezes, N.A. (2016) Biogeografia dos Peixes de Água Doce da América do Sul. *In*: Carvalho, C.J.B. & Almeida, E.A.B. (2Ed), *Biogeografia da América do Sul, análise de tempo, espaço e forma*. Rio de Janeiro, 298p. 245–258.

Roxo, F.F. Oliveira, C. & Zawadzki, C.H. (2012a) Three new species of the *Neoplecostomus* (Teleostei: Siluriformes: Loricariidae) from the Upper Rio Paraná basin of southeastern Brazil. Zootaxa, 3233, 1–21.

Roxo, F.F., Zawadzki, C.H., Costa-Silva, G.J., Chiachio, M.C., Foresti, F. & Oliveira, C. (2012b) Molecular systematics of the armored neotropical catfish subfamily Neoplecostominae (Siluriformes, Loricariidae) *Zootaxa,* 3390, 33–42.

Roxo, F.F., Silva, G.S.C., Zawadzki, C.H. & Oliveira, C. (2014a) *Neoplecostomus doceensis*: a new loricariid species (Teleostei, Siluriformes) from the Rio Doce basin and comments about its putative origin. *ZooKeys*, 440, 115–127.

Roxo, F.F., Albert, J.S., Silva, G.S., Zawadzki, C.H., Foresti, F. & Oliveira, C. (2014b) Molecular Phylogeny and Biogeographic History of the Armored Catfish Subfamilies Hypoptopomatinae, Neoplecostominae and Otothyrinae (Silurifomes: Loricariidae), *PlosOne*, 9 (8), 1–17.

Roxo, F.F., Ochoa, L.E., Costa-Silva, G.J. & Oliveira, C. (2015) Species delimitation in *Neoplecostomus* (Siluriformes: Loricariidae) using morphologic and genetic approaches. *DNA Barcodes*, 15, 110–117.

Roxo, F.F., Silva, G.S.C., Zawadzki, C.H. & Oliveira, C. (2017) *Neoplecostomus canastra,* a new catfish (Teleostei: Siluriformes) species from upper Rio Paraná basin. *Zootaxa*, 4294(2), 226–240.

Silva, G.S.C., Reia, L., Zawadzki, C.H. & Roxo, F.F. (2019) New species of *Neoplecostomus* (Neoplecostomini: Loricariidae) lacking adipose fin from upper Rio Paraná basin, central Brazil. *Zootaxa*, 4544, 093–102.

Stamatakis, A. (2014) RaxML Version 8: A tool for phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics*, 9(9), 1312–1313.

Steindachner, F. (1877) Die Süsswasserfische des südöstlichen Brasilien (III). *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe*, 74, 559–694.

Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0., *Molecular Biology and Evolution*, 30(12), 2725–2729.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Herbert, P.D.N. (2005) DNA barcoding Australia´s fish species. *Philosophical Transactions of the Royal Society B*, 360, 1847–1857.

Zawadzki, C.H., Alves, A.L., Renesto, E. & Oliveira, C. (2004) Biochemical evidence of a possible new species of *Neoplecostomus* (Teleostei: Loricariidae) from the Upper Rio Paraná basin, Brazil. *Biochemical Systematics and Ecology*, 32, 573–582.

Zawadzki, C.H., Pavanelli, C.S. & Langeani, F. (2008) *Neoplecostomus* (Teleostei: Loricariidae) from the Upper Rio Paraná basin, Brazil, with description of three new species. *Zootaxa*, 1757, 31–48.