UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS HUMANAS E BIOLÓGICAS
DEPARTAMENTO DE BIOLOGIA
BACHARELADO EM CIÊNCIAS BIOLÓGICAS

BEATRIZ MARTINS CORAZZA

CARACTERIZAÇÃO MORFOLÓGICA DO PEIXE CACHIMBO *MICROPHIS LINEATUS* (KAUP 1856) NA COSTA BRASILEIRA (TELEOSTEI, SYNGNATHIFORMES, SYNGNATHIDAE)

SOROCABA - SP
2021

UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS HUMANAS E BIOLÓGICAS
DEPARTAMENTO DE BIOLOGIA
BACHARELADO EM CIÊNCIAS BIOLÓGICAS

BEATRIZ MARTINS CORAZZA

CARACTERIZAÇÃO MORFOLÓGICA DO PEIXE CACHIMBO *MICROPHIS LINEATUS* (KAUP 1856) NA COSTA BRASILEIRA (TELEOSTEI, SYNGNATHIFORMES, SYNGNATHIDAE)

Trabalho de Conclusão de Curso apresentado ao Curso de Bacharelado em Ciências Biológicas da Universidade Federal de São Carlos *campus* Sorocaba para obtenção do título de Bacharela em Ciências Biológicas.

Orientação: Prof. Dr. George Mendes Taliaferro Mattox.

SOROCABA - SP

2021

Corazza, Beatriz Martins

Caracterização morfológica do peixe cachimbo *Microphis lineatus* (Kaup 1856) na costa brasileira (Teleostei, Syngnathiformes, Syngnathidae) / Beatriz Martins Corazza -- 2021.
58f.

TCC (Graduação) - Universidade Federal de São Carlos, campus Sorocaba, Sorocaba
Orientador (a): George Mendes Taliaferro Mattox
Banca Examinadora: Eliane Pintor de Arruda, Priscila Camelier de Assis Cardoso
Bibliografia

1. Caracteres merísticos. 2. Morfometria. 3. Variação clinal. I. Corazza, Beatriz Martins. II. Título.

Ficha catalográfica desenvolvida pela Secretaria Geral de Informática (SIn)

DADOS FORNECIDOS PELO(A) AUTOR(A)

Bibliotecário(a) responsável: Maria Aparecida de Lourdes Mariano – CRB/8 6979

Folha de aprovação

Beatriz Martins Corazza

"Caracterização morfológica do peixe cachimbo *Microphis lineatus* (Kaup 1856) na costa brasileira (Teleostei, Syngnathiformes, Syngnathidae)"

Trabalho de Conclusão de Curso

Universidade Federal de São Carlos – Campus Sorocaba

Sorocaba, 5 de janeiro de 2021.

Orientador _____
Prof. Dr. George Mendes Taliaferro Mattox

Membro 1 _____
Profa. Dra. Eliane Pintor de Arruda

Membro 2 _____
Profa. Dra. Priscila Camelier de Assis Cardoso

Para a minha família e, em especial, à minha avó Dóra (*in memorian*), a minha fã número 1

“I know of no pleasure deeper than that which comes from contemplating the natural world and trying to understand it.”

— Sir David Attenborough, *Life on Air* (2002)

AGRADECIMENTOS

Agradeço aos professores, técnicos e servidores da UFSCar *campus* Sorocaba por todo conhecimento, suporte e atenção. Aos meus amigos e colegas de sala pela união, colaboração e troca durante esses anos de graduação. Um obrigada cheio de carinho a minha dupla desde o primeiro dia de aula, Jéssica, por todos os momentos e experiências vivenciadas.

Agradeço imensamente as diversas oportunidades, conversas, orientações e conhecimentos pacientemente compartilhados e proporcionados pelo meu queridíssimo orientador Prof. Dr. George Mattox. Uma inspiração de pesquisador, orientador, professor e pessoa, que certamente me proporcionou vivências que levarei com carinho para o resto da vida. Agradeço também pela confiança e incentivo em todos os momentos.

Agradeço do fundo do coração a minha família e, especialmente, ao meu núcleo familiar, meus pais Marilena e José Alberto, minha irmã Lívia e a minha avó Dóra (*in memorian*) por sempre me apoiarem e acreditarem em mim e, independente da minha escolha, sempre me ensinaram a colocar um peso maior naquilo que me faz feliz e faz sentido para mim.

Sou muito grata pelas pessoas maravilhosas que fizeram do laboratório a minha segunda casa, tanto integrantes do LISO quanto do LEPBio: George, Karolina, Péricles, Caio, Gabriela, Yasmin, Bianca, Haíla, Esteban, Eduardo, Silvio, Júlia, Gabriel Baréa, Gabriel Romano, Thaís, Guilherme, Pietro. Obrigada pela convivência, pelos cafés, conselhos, risadas, colaboração, aprendizados, trocas, enfim, obrigada por me permitirem crescer de várias formas e jeitos. Agradeço à Júlia d'Oliveira pela lindíssima ilustração da espécie estudada presente na "Introdução ao Trabalho".

Agradeço aos meus amigos Caroline, Gabriela, Amanda e Ryu por sempre torcerem por mim e acompanharem-me em várias fases da minha vida. As minhas amizades construídas em congressos, Polônia, Letícia, Matheus e Felipe, minhas conexões em Minas Gerais e João Pessoa.

Agradeço à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pelo financiamento concedido ao desenvolvimento deste projeto (Proc. 2019/10894-5) e, principalmente, pelo incentivo a Ciência básica.

Por fim, com receio de não ter incluído todas as pessoas que eu sou grata, deixo o meu último agradecimento a todos aqueles que passaram e, especialmente, aos que ficaram durante essa jornada da graduação.

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

RESUMO

Syngnathidae inclui cavalos-marinhos, dragões marinhos e peixes cachimbo, como a espécie *Microphis lineatus*, que ocorre em água doce, estuarina e marinha na costa Atlântica, dos EUA ao Brasil, e Pacífica do Panamá na região do canal. Apresenta incerteza taxonômica sendo reconhecida como espécie por alguns e subespécie por outros autores. Há 40 anos, foi observada variação clinal de três dados merísticos na costa brasileira, com contagens aumentando no sentido norte-sul. O material disponível na época, no entanto, era restrito para explorar estas clinas detalhadamente. Este estudo teve o objetivo de caracterizar morfologicamente *M. lineatus* na costa brasileira, possibilitando explorar tais clinas com material acumulado neste período, além de fornecer subsídios para futura revisão taxonômica da espécie. Tomaram-se dezessete caracteres morfométricos e doze merísticos de 209 exemplares provenientes de seis coleções ictiológicas brasileiras. As três características com suposta clina foram corroboradas (número de raios da nadadeira dorsal, total de anéis subdorsais e número de anéis da cauda). A análise de simetria das nadadeiras peitorais com as contagens de seus raios evidenciou a inexistência de assimetria bilateral nesta característica. Propõe-se aqui um novo limite austral para a distribuição de *M. lineatus*: Rio Grande do Sul.

Palavras-chave: caracteres merísticos, morfometria, variação clinal.

ABSTRACT

Syngnathidae includes seahorses, sea dragons and pipefishes, such as the species *Microphis lineatus*, which occurs in fresh, estuarine and marine waters off the Atlantic coast, from the USA to Brazil, and Pacific coast of Panama in the canal zone. It presents taxonomic uncertainty and is recognized as a species by some researchers and subspecies by others. Forty years ago, a clinal variation of three meristic data on the Brazilian coast was observed, with counts increasing in the north-south direction. The relatively small amount of material available at the time, however, hindered a better understanding of these clines. This study aimed to morphologically characterize *M. lineatus* in the Brazilian coast, making it possible to explore such clines with material accumulated in this period, in addition to providing subsidies for a future taxonomic review of the species. Seventeen morphometric and twelve meristic characters were taken from 209 specimens from six Brazilian ichthyological collections. The three characteristics with putative clines were corroborated (number of dorsal-fin rays, total number of subdorsal rings and number of tail rings). The symmetry analysis of the pectoral fins with the counting of their rays showed the absence of bilateral asymmetry in this characteristic. A new southern limit for the distribution of *M. lineatus* is proposed here: Rio Grande do Sul.

Keywords: meristic characters, morphometry, clinal variation.

1. INTRODUÇÃO AO TRABALHO

O manuscrito foi elaborado seguindo as regras de formatação da revista Neotropical Ichthyology (ANEXO A) visando a sua futura submissão.

ANEXO B

2. ARTIGO

Morphological characterization of the pipefish *Microphis lineatus* (Kaup, 1856) along the Brazilian coast (Syngnathiformes: Syngnathidae)

Beatriz Martins Corazza[1] and George Mendes Taliaferro Mattox[1]

[1] Laboratório de Ictiologia de Sorocaba (LISO), Departamento de Biologia, Universidade Federal de São Carlos – Campus Sorocaba, Rod. João Leme dos Santos, km 110, Itinga, 18052-780 Sorocaba, SP, Brazil.. (BMC) beamcorazza@gmail.com, (GMTM) gmattox@ufscar.br; https://orcid.org/0000-0003-4748-472X (corresponding author)

Abstract

Syngnathidae includes seahorses, sea dragons and pipefishes, such as the species *Microphis lineatus*, which occurs in fresh, estuarine and marine waters off the Atlantic coast, from the USA to Brazil, and Pacific coast of Panama in the canal zone. It presents taxonomic uncertainty and is recognized as a species by some researchers and subspecies by others. Forty years ago, a clinal variation of three meristic data on the Brazilian coast was observed, with counts increasing in the north-south direction. The relatively small amount of material available at the time, however, hindered a better understanding of these clines. This study aimed to morphologically characterize *M. lineatus* in the Brazilian coast, making it possible to explore such clines with material accumulated in this period, in addition to providing subsidies for a future taxonomic review of the species. Seventeen morphometric and twelve meristic characters

were taken from 209 specimens from six Brazilian ichthyological collections. The three characteristics with putative clines were corroborated (number of dorsal-fin rays, total number of subdorsal rings and number of tail rings). The symmetry analysis of the pectoral fins with the counting of their rays showed the absence of bilateral asymmetry in this characteristic. A new southern limit for the distribution of *M. lineatus* is proposed here: Rio Grande do Sul.

Keywords: meristic characters, morphometry, clinal variation.

Resumo

Syngnathidae inclui cavalos-marinhos, dragões marinhos e peixes cachimbo, como a espécie *Microphis lineatus,* que ocorre em água doce, estuarina e marinha na costa Atlântica, dos EUA ao Brasil, e Pacífica do Panamá na região do canal. Apresenta incerteza taxonômica sendo reconhecida como espécie por alguns e subespécie por outros autores. Há 40 anos, foi observada variação clinal de três dados merísticos na costa brasileira, com contagens aumentando no sentido norte-sul. O material disponível na época, no entanto, era restrito para explorar estas clinas detalhadamente. Este estudo teve o objetivo de caracterizar morfologicamente *M. lineatus* na costa brasileira, possibilitando explorar tais clinas com material acumulado neste período, além de fornecer subsídios para futura revisão taxonômica da espécie. Tomaram-se dezessete caracteres morfométricos e doze merísticos de 209 exemplares provenientes de seis coleções ictiológicas brasileiras. As três características com suposta clina foram corroboradas (número de raios da nadadeira dorsal, total de anéis subdorsais e número de anéis da cauda). A análise de simetria das nadadeiras peitorais com as contagens de seus raios evidenciou a inexistência de assimetria bilateral nesta característica. Propõe-se aqui um novo limite austral para a distribuição de *M. lineatus*: Rio Grande do Sul.

Palavras-chave: caracteres merísticos, morfometria, variação clinal.

2.1. Introduction

2.1.1. Group and species biology

Syngnathidae includes seahorses, seadragons and pipefishes. It is a family of charismatic, diverse and morphologically specialized fish, composed of more than 300 valid species and 57 genera, with pipefishes represented by 54 genera, distributed in marine, estuarine and freshwater habitats (Wilson & Orr, 2011; Nelson et al., 2016; Dallevo-Gomes et al., 2020; Fricke et al., 2020).

Syngnathids are predators that feed mainly by sucking small crustaceans, like amphipods and copepods (Kuiter, 2009; Manning et al., 2019). Some general characteristics of the family are the thin and elongate body covered by bony plates that form a series of bony rings and ridges, gill opening reduced to a pore in the opercular membrane, small and terminal mouth with fused and toothless jaws and absence of pelvic fin (Dawson, 1982; Manning et al., 2019).

The group has an unusual characteristic related to reproduction and sexual dimorphism. Mature males are responsible for incubating eggs in the ventral portion of their bodies and, depending on the species, eggs can be found exposed, partially or completely covered in a brood pouch (Dawson, 1982; Wilson & Orr, 2011). The development and location of the brood pouch and the laying of eggs in males were frequently used to classify Syngnathidae. Dunker (1912, 1915) divided the family into two groups, the Gastrophori and Urophori, which correspond respectively to the laying of eggs in the ventral part of the trunk or tail (Dawson, 1982).

The economic interest of Syngnathidae is not usually as a source of animal protein as in many other teleosts, but rather they are attractive to aquarists and in some regions they are dried or incorporated in plastic to be sold as *souvenirs*, or used in traditional medicine (Dawson, 1982; Kuiter, 2009; Vincent et al., 2011).

The genus *Microphis* Kaup belongs to the subfamily Nerophinae, characterized by a brood pouch present on the trunk (= Gastrophori) (Hamilton et al., 2017; Fricke et al., 2020). The genus typically has 9 rays in the caudal fin, a ridge above the gill opening, well developed brood pouch plates and does not present dermal flaps (Dawson, 1984).

The species *Microphis lineatus* (Kaup 1856) (Fig. 1), also known as the Opossum River Pipefish (Pollom, 2015), presents a unique combination of characters among the species of the Western Atlantic, with 17 to 23 rays in the pectoral fin and convergent inferior trunk and tail

ridges. The length of the specimens rarely exceeds 200 mm Standard Length (SL), with the smallest mature male analysed by Dawson (1982) being 91.5 mm SL (Dawson, 1982).

FIGURE 1

Microphis lineatus is distributed throughout the Western Atlantic Ocean, with records ranging from New Jersey to the state of São Paulo and in the Pacific terminal of the Panama Canal (Dawson, 1982, 1984). It occurs in fresh, estuarine and marine waters, but reproductive individuals tend to be found in bodies of fresh water or low salinity, while euryhaline young can be found in the three habitat types (Dawson, 1982, 1984; Smith-Vaniz & Jelks, 2014). They occupy environments with varied substrates, from mangroves to very vegetated streams. The individuals collected in marine waters were found from close to the surface to depths of 183 m or more, while in islands and continental environments they were found in waters up to 3 meters deep (Dawson, 1982).

2.1.2. Taxonomic history of the species

The species currently known as *Microphis lineatus* was initially described by Kaup (1856) as *Doryichthys lineatus*, characterized by a yellowish-brown base color, three silvery stripes along the lateral line and three to five black spots on the lower part of the snout, mainly seen in well-preserved specimens. Despite having indicated syntypes from lots with specimens collected in Bahia, México and Guadalupe from the collection of the Muséum National d'Histoire Naturelle, Paris, a holotype was not attributed to the species by Kaup (1856). Later the species lectotype (MNHN 6213 – presumably a female – Veracruz, Mexico) was selected by Dawson (1979, 1984).

Kaup (1856) introduced the names of the genera *Doryichthys* and *Microphis*, but did not clearly differentiate them (Dawson, 1979). Hubbs (1929) created the genus *Oostethus* to group the species typified by *Doryichthys lineatus* Kaup and *Syngnathus brachyurus* Bleeker. Previously, both species had been allocated to the genus *Microphis* (non Kaup) by Duncker (1910) who employed the name of the genus created by Kaup to a new genus (Hubbs, 1929; Dawson, 1979).

Doryichthys lineatus came to be classified as *Oostethus brachyurus lineatus*, one of the four subspecies of *Oostethus brachyurus* (Bleeker, 1853) separated from allopatric populations

of the species, according to the work of Mayr (1969) (Dawson, 1979). Dawson (1979) mentioned that the four subspecies were treated as three species by some authors, among them *Oostethus lineatus* (Kaup, 1856). Subsequently, Dawson (1984) started to recognize *Oostethus brachyurus lineatus* as the only one of the four subspecies of the polytypic species *Microphis (Oostethus) brachyurus* Bleeker that occurs in the Western Atlantic, known as *Microphis (Oostethus) brachyurus lineatus* (Kaup) (Dawson, 1984; Smith-Vaniz & Jelks, 2014).

The current name of the species is *Microphis lineatus* (Kaup 1856) and it was used in Ferraris's catalog (Reis et al., 2003) as a small change in the nomenclature of Dawson's works (1982, 1984), however it was emphasized that the names *Oostethus lineatus* and *Microphis brachyurus lineatus* are still used in recent literature (Ferraris in Reis et al., 2003; Angulo et al., 2013). *Microphis lineatus* (Kaup 1856) currently has the following species as junior synonyms: *Syngnathus cayennensis* Sauvage 1882, *Doryichthys dumerili* Kaup 1856, *Siphostoma brevicaudum* Meek 1904, *Siphostoma torrei* Nichols 1912, *Doryrhamphus sierra* Nichols 1915 and *Siphostoma eigenmanni* Wilson 1916 (Fricke et al., 2020).

2.1.3. Observed clinal variation

Dawson (1979, 1982) indicated that the counts of number of tail rings, number of dorsal-fin rays and total number of subdorsal rings for Brazilian specimens of the species *Microphis lineatus* from states further north were similar to the counts of Caribbean specimens and tended to increase their values when found further south of Brazil, pointing out the possible clinal variation for these characters. Dawson (1982) evidenced the data limitation, mainly for the states in the north of Brazil, as the amount of material available was less than presently. The variation of some characteristics, especially the count of the tail rings, was not clear for Brazilian specimens (Dawson, 1982). The aim of this paper was to characterize *M. lineatus* morphologically in the Brazilian coast and access the clinal variation observed by Dawson with material accumulated in the last 40 years, in addition to provide subsidies for a future taxonomic review of the species.

2.2.Material and Methods

Material examined was obtained from the following institutions: Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso" (ZUEC), Museu de Zoologia da Universidade Estadual de Feira de Santana (MZFS), Museu de Zoologia da Universidade Estadual de Londrina (MZUEL), Museu de Zoologia da Universidade de São Paulo (MZUSP), Universidade Federal da Bahia (UFBA) and Universidade Federal do Rio Grande do Sul (UFRGS).

Sex was determined by the presence of the brood pouch in mature males, the other specimens being considered as females and juveniles. Measurements were taken with a digital caliper with precision of 0.1 mm, while the meristic data were counted with the aid of a stereomicroscope. Figure 1 was made with a Zeiss Discovery V20 stereomicroscope with a Zeiss Axiocam digital camera attached. All observations were made on the left side of the body, whenever possible, and were based on the methodology of Dawson (1982), Lourie & Randall (2003) and Dallevo-Gomes et al. (2020).

Some characteristics were also observed, such as the color of specimens, the presence of brown spots on the snout, the configuration of the lateral ridges, the presence of dorsal, pectoral and anal fins and the presence of ridges and dermal flaps in the head, count of bone rings in the body and fin rays, based on the methodology of Dawson (1982).

Measurements and meristic counts are outlined in Dallevo-Gomes et al. (2020). The 17 morphometric characters are: standard length (SL), head length (HL), snout length (SL), snout depth (SD), snout width (SW), orbital diameter (OD), post-orbital length (POL), head depth (HD), pectoral-fin length (PFL), pectoral-fin base length (PBL), dorsal-fin base length (DBL), anal ring depth (AD), trunk depth (TD), body depth (BD), body width (BW), tail length (TL), brood pouch length (BPL); and the 12 meristic characters are: number of trunk rings (NTR), number of tail rings (NLR), number of brood pouch rings (NBR), total number of subdorsal rings (TNSR), total number of body rings (TNBR), point of origin of dorsal-fin (POD), number of pectoral-fin rays (both sides) (NPRL and NPRR), number of dorsal-fin rays (NDR), number of caudal-fin rays (NCR), number of anal-fin rays (NAR), number of longitudinal rows of eggs (NLRE). For the measures, subunits of the body were presented as percentages of SL, and subunits of the head were presented as percentages of HL. Some specimens were not considered in certain statistical analysis because they did not present a reliable standard length measurement, since they were broken or had malformations in the trunk or caudal portion of the body. The symmetry analysis of the left and right pectoral fins was based on the mode, range, mean and standard deviation of their ray counts.

The meristic data were organized in tables of frequency distribution according to the order of Brazilian coastal states in the north-south direction. The morphometric data were organized in a table containing the range, mean and standard deviation of the relative values of the subunits of the head in relation to the length of the head and of the subunits of the body in relation to the standard length. The only absolute measurement is the standard length.

To investigate the clinal variation of the meristic data mentioned by Dawson (1979) (NDR, TNSR and NLR), the Mantel test was applied considering the Pearson correlation coefficient at the 5% significance level ($p < 0.05$) using R version 4.0.3 (R core Team, 2020). The possible correlation between the meristic data variation and the analysed lots collecting points locations, represented by the variations of latitude (°) and variations of geographic distance between the coordinate points (km), was tested.

2.3. Results

Forty-nine lots containing 209 specimens from six collections were examined. Information on the study material can be found in the **S1**. The lots were organized by the order of Brazilian coastal states in the north-south direction. For each lot, collection acronym and catalog number, number of specimens examined, standard length range (mm SL), collection site, collectors and date of collection were provided.

The collection locations of the Brazilian lots analysed is presented in Fig. 2 and the number of specimens analysed by state in Fig. 3. Of the 209 specimens, 149 were identified as juveniles and/or females and 60 as mature males (Fig. 4).

FIGURE 2

FIGURE 3

FIGURE 4

The meristic data collected are present in Tabs. 1 – 12 and the morphometric data in Tab. 13. The values obtained by the Mantel test considering the correlation with latitude variations (°) and the counts of the dorsal-fin rays (NDR), were: $p = 0.0001$, $r = 0.3107$ and $r^2 = 0.0965$; for the counts of the total number of subdorsal rings (TNSR) the values obtained were: $p = 0.0001$, $r = 0.4306$, $r^2 = 0.1884$; and for the number of tail rings (NLR) the values obtained were: $p = 0.0421$, $r = 0.1012$, $r^2 = 0.0102$. For the correlation between the variation of

the geographical distance (km) and NDR counts, the results were: p = 0.0001, r = 0.2943 and r^2 = 0.0866; for the TNSR counts the values obtained were: p = 0.0001, r = 0.403, r^2 = 0.1624; and for the NLR counts the values obtained were: p = 0.0438; r = 0.09846; r^2 = 0.0097. Graphs generated by the analyses are presented in Fig. 5.

The values resulting from the Mantel test (Fig. 5) show that a positive and statistically significant correlation (p <0.05) between the number of dorsal-fin rays (NRD), the total number of subdorsal rings (TNSR) and the number of tail rings (NLR) with the variation in distance between the specimen collecting points, considering both variations in latitude (°) and geographic distance between coordinate points (km). Approximately 10% of the variation in the NDR values can be explained by the variation in latitude and approximately 9% by the variation in geographical distance. For TNSR values, approximately 19% of its variation can be explained by the variation in latitude and approximately 16% by the variation of the geographical distance. Finally, for NRL values, approximately 1% of its variation can be explained by the variation in latitude and the same value was observed for the variation of the geographical distance.

TABLES 1 – 13

FIGURE 5

The symmetry analysis of the pectoral fins using their ray counts can be seen in Tab. 14. The analysis showed that the species does not present bilateral asymmetry for this characteristic.

TABLE 14

Fourteen specimens showed malformations in the caudal rings, especially in the posterior ones. Most of them (12 of the 14 specimens) have counts that vary between 6 – 13 caudal rays and are different from 9 rays, which is considered a diagnostic character for the species.

2.4. Discussion

In the frequency distribution tables, the characters “number of dorsal-fin rays” (Tab. 1) and “total number of subdorsal rings” (Tab. 2) seem to show a clinal variation in their values,

which is consistent to the result of Mantel test. The cline was not clear looking at the table for the characteristic "number of tail rings" (Tab. 3). Probably due to the gap or lack of data for some states, such as Espírito Santo, the correlation between NLR and the variations in latitude (°) and geographic distance (km) was relatively low; another reason that may have influenced the result of this correlation was the reduction of the sample size for the characteristic NLR, due to the exclusion of specimens with malformations in the tail in analyses.

Clinal variation consists of the gradual variation of a characteristic along the distribution of a species and they are known for morphometric and meristic characters in fish, such as number of pored scales in lateral line, number of branchial spines on the first gill arch, number of transverse scale rows and number of fin rays, that are correlated with latitude and, in some cases, with longitude (Holčík & Jedlička, 1994; Ivankov et al., 2013). Barlow (1961) mentions in his work that geographic clines can reflect adaptive changes of a genetic nature.

Variability for meristic counts between populations tends to be greater and more evident in fish that spawn their eggs into the environment, than those that lay their eggs in brood pouches, as in the case of syngnathids. This may be due to the relatively exposition of the eggs to the environmental conditions, such as salinity, dissolved oxygen concentration and mainly temperature. Conditions that slow down the metabolism and development of organisms, especially in the embryonic and larval stages of fish, are expected to generate higher counts of meristic characters (Barlow, 1961; Ivankov et al., 2013).

Despite the above observations, the occurrence of clinal variation is frequently observed in members of the Syngnathidae family. Intraspecific tendencies are not considered unusual, either related to longitude (west-east), as it is observed for counts in the number of tail rings and the number of dorsal-fin rays in some species of pipefish from the Indo-Pacific such as *Nannocampus pictus* (Duncker 1915), or with latitude (north-south), as observed for Brazilian specimens of the species *Microphis lineatus* for the characteristics NRD, NLR and TNSR presented herein (Dawson, 1977, 1979, 1982) and *Pseudophallus brasiliensis* (Dallevo-Gomes et al., 2020).

Dawson (1982) mentioned that the number of caudal-fin rays is a highly conservative character, therefore, values other than 9 rays indicate regeneration, which can lead to atypical counts of the number of tail rings. However, six specimens with caudal-fin rays counts other than 9 from the states of Rio Grande do Norte, Bahia and Rio de Janeiro (Tab. 10) were kept in the analysis, as they did not appear to present abnormalities.

The states of Maranhão, Piauí, Ceará, Paraíba, Pernambuco, Paraná and Santa Catarina did not have specimens analysed. This may reflect the absence of records of *Microphis lineatus* in part of these states (*e.g.* Paraná and Santa Catarina), absence of lots records from some of these states in the *speciesLink* system (*e.g.* Pernambuco and Ceará), and, mainly due to the COVID-19 pandemic, which made it impossible to borrow new lots from some institutions (*e.g.* Universidade Estadual do Piauí – UESPI, Piauí, Universidade Federal da Paraíba – UFPB, Paraíba, Instituto Nacional da Mata Atlântica – INMA, Espírito Santo and Museu de Ciências e Tecnologia – MCP, Rio Grande do Sul, with all of these loans being suspended). This was reflected in the low sampling of specimens from Espírito Santo, for instance. All these institutions have lots putatively identified as *M. lineatus* but, for now, it is not possible to know whether the lots not analysed correspond indeed to *M. lineatus*.

Based on the *speciesLink* system, the contact with curators of some of the scientific collections, and the work of Dawson (1982), it seems like some states do not have many specimens of *Microphis lineatus* sampled, as is the case of the states of Maranhão, Piauí, Ceará and Pernambuco. This may indicate that *M. lineatus* is rare in these regions or even that it was not much collected from the coastal water bodies in these states. The states of Paraná and Santa Catarina do not have known lots, however two specimens from Rio Grande do Sul were analysed (UFRGS 3646 and UFRGS 3647), which indicate an expansion in the distribution of the species. Thus, the southern limit of the species on the west coast of the Atlantic is no longer the state of São Paulo, but the state of Rio Grande do Sul. A similar southern expansion of the distribution range was registered for another pipefish species, *Pseudophallus brasiliensis*, which was known to occur only to the state of São Paulo until Dallevo-Gomes et al. (2020) recorded specimens from Paraná and Santa Catarina, the new austral limit of the distribution of that species.

Microphis lineatus does not have enough data to assess its level of conservation by the International Union for Conservation of Nature (IUCN), and is currently categorized as "Data Deficient" (DD). What justifies the category is that the species is widely distributed, there is speculation that freshwater specimens could be considered as a separate species and the species occupation habitats, mainly the freshwater localities, are susceptible to degradation in some locations, but the level of global threat is unknown (Pollom, 2015). The Brazilian specimens analysed did not appear to present significant morphological differentiation for the different collection sites environments, but only a broad taxonomic study of *Microphis lineatus* from the

whole distribution area of the species will cast light on its identity and, consequently, yield more data on its conservation.

2.5. Acknowledgments

We thank Osvaldo T. Oyakawa (MZUSP) and Flávio C. T. Lima (ZUEC) for granting access to the ichthyological collections, for the helpful advices and for loan of material. For the loan of material, we also acknowledge Angela M. Zanata and Priscila Camelier (UFBA), Juliana M. Wingert and Luiz R. Malabarba (UFRGS), José L. Birindelli (MZUEL) and Michel D. Gianeti (MZUSP). We acknowledge Caio I. A. Dallevo-Gomes (IBUSP) for helping with the methodology, advices and knowledge about pipefishes, Maurício Cetra (UFSCar) for the indication of the Mantel test to investigate the possible clinal variations and Gabriel E. B. de Barros (FFCLRP - USP) for helping to apply the Mantel test and use of R language. Financial support was provided by the Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP (Proc. 2019/10894-5) to BMC. This study also benefitted from equipment acquired by FAPESP (Proc. 2017/01970-4).

2.6. Literature cited

Angulo A, Garita-Alvarado CA., Bussing WA, López MI. Annotated checklist of the freshwater fishes of continental and insular Costa Rica: additions and nomenclatural revisions. Check List, 2013; 9(5): 987–1019. http://dx.doi.org/10.15560/9.5.987.

Barlow GW. Causes and Significance of Morphological Variation in Fishes. Syst Biol, 1961; 10(3): 105–117. https://doi.org/10.2307/2411595.

Dallevo-Gomes CIA, Mattox GMT, Toledo-Piza M. Taxonomic review of the pipefish genus *Pseudophallus* Herald, with the description of a new species (Syngnathiformes: Syngnathidae). Zootaxa, 2020; 4859(1):81–112. https://doi.org/10.11646/zootaxa.4859.1.3.

Dawson CE. Synopsis of syngnathine pipefishes usually referred to the genus *Ichthyocampus* Kaup, with description of new genera and species. Bull Mar Sci, 1977; 27(4): 595–650. Available from: https://www.ingentaconnect.com/content/umrsmas/bullmar/1977/00000027/00000004/art00001.

Dawson CE. Review of the polytypic Doryrhamphine pipefish *Oostethus brachyurus* (Bleeker). Bull Mar Sci, 1979; 29(4): 465–80. Available from: https://www.ingentaconnect.com/content/umrsmas/bullmar/1979/00000029/00000004/art00003#.

Dawson CE. Family Syngnathidae. The Pipefishes (subfamilies Doryrhamphinae and Syngnathinae). In: Dawson CE, Vari RP. Fishes of the Western North Atlantic. New Haven: Sears Foundation for Marine Research, Yale Univ.;1982. 1(8): 1–172.

Dawson CE. Revision of the genus *Microphis* Kaup (Pisces, Syngnathidae). Bull Mar Sci, 1984; 35(2): 117–181. Available from: https://www.ingentaconnect.com/content/umrsmas/bullmar/1984/00000035/00000002/art00001.

Fricke R, Eschmeyer WN, Van der Laan R. Eschmeyer's catalog of fishes: genera, species, references [Internet]. San Francisco: California Academy of Science; 2020. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

Hamilton HN, Saarman GS, Sellas AB, Moore B, Hoang T, Grace CL, Gomon M, Crow K, Simison WB. Molecular phylogeny and patterns of diversification in syngnathid fishes. Mol Phylogenet Evol, 2017; 107: 388–403. https://doi.org/10.1016/j.ympev.2016.10.003.

Holčík J, Jedlička L. Geographical variation of some taxonomically important characters in fishes: the case of the bitterling *Rhodeus sericeus*. Environ Biol Fishes, 1994; 41: 147–170. https://doi.org/10.1007/BF00023810.

Hubbs CL. *Oostethus*: a new generic name for a doryrhamphine pipefish. Occas Pap Mus Zool Univ Mich, 1929; 199: 1–4. Available from: http://hdl.handle.net/2027.42/56638.

Ivankov VN, Ivankova EV, Borisovets EE, Vyshkvartsev DI. Interpopulation Morphological Variability of the Viviparous Pipefish *Syngnathus acusimilis* Günther, 1873 (Teleostei: Syngnathidae). Russ J Mar Biol, 2013; 39(5): 340–49. https://doi.org/10.1134/S1063074013050039.

Kaup JJ. Catalogue of lophobranchiate fish in the collection of the British Museum. London: Printed by order of the Trustees; 1856.

Kuiter RH. Seahorses and their relatives. Seaford, Austrália: Aquatic Photographics; 2009.

Lourie SA, Randall JE. A new pygmy seahorse, *Hippocampus denise* (Teleostei: Syngnathidae), from the Indo-Pacific. Zool Stud, 2003; 42(2): 284–291. Available from: http://zoolstud.sinica.edu.tw/Journals/42.2/284.pdf.

Manning CG, Foster SJ, Vincent CJ. A review of the diets and feeding behaviours of a family of biologically diverse marine fishes (Family Syngnathidae). Rev Fish Biol Fish, 2019; 29:197–221. https://doi.org/10.1007/s11160-019-09549-z(0123456789.

Nelson JS, Grande TC, Wilson MVH. Fishes of the world (5th Edition). New York: John Wiley and Sons; 2016.

Pollom R. *Microphis brachyurus* ssp. *lineatus* [Internet]. The IUCN Red List of Threatened Species, 2015. Available from: https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T46108938A46959096.en.

R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.R-project.org/.

Reis RE, Kullander SO, Ferraris CJ, Jr., editors. Check list of the freshwater fishes of South and Central America. Porto Alegre: Edipucrs; 2003.

Smith-Vaniz W, Jelks HL. Marine and inland fishes of St. Croix, U. S. Virgin Islands: an annotated checklist. Zootaxa, 2014; 3803(1): 001–120. http://dx.doi.org/10.11646/zootaxa.3803.1.1.

Vincent ACJ, Foster SJ, Koldewey HJ. Conservation and management of seahorses and other Syngnathidae. J Fish Biol, 2011; 78: 1681–1724. https://doi.org/10.1111/j.1095-8649.2011.03003.x.

Wilson AB, Orr JW. The evolutionary origins of Syngnathidae: pipefishes and seahorses. J Fish Biol, 2011; 78: 1603-1623. https://doi.org/10.1111/j.1095-8649.2011.02988.x.

2.7.Supplementary files

S1. List of the Study Material. Lots were organized by the order of Brazilian coastal states in the north-south direction and include: collection acronym and catalog number, number of specimens examined, standard length range (mm SL), collection site, collectors and date of collection were provided for each lot.

Brasil: Amapá: ZUEC PIS 16504, 3, 76.8 – 85.8 mm SL, Igarapé Serrapi, tributary of Rio Oiapoque above Clevelândia do Norte, Oiapoque, 03°48'21"N 51°51'58"W, T. C. Pessali, T. C. Faria & E. Neuhaus, 4 Oct 2017; **Pará:** MZUSP 10276.0, 1, 160.8 mm SL, Iguarapé Uruazinho, Vila Maiauatá, Expedição Permanente à Amazônia, 27 Aug 1970; MZUSP 10277.0, 1, 148.5 mm SL, Igarapé do grilo, Rio Tocantins, Expedição Permanente à Amazônia, 4 Sept 1970; **Rio**

Grande do Norte: MZUEL 03018, 10, 99.2 – 148.5 mm SL, Tibau do Sul, Lisandro Juno, Marcelo, Márcia, Neto, Débora & Andéa, 25 Nov 1990; MZUEL 03019, 1, 148.0 mm SL, Rio Pium, Parnamirim, northeast drainage, V. Bháskara Canan, 29 Nov 1992; MZUEL 03020, 1, 99.4 mm SL, , Rio Pium, Parnamirim, northeast drainage, Bháskara Canan & Lisandro Juno, 24 Jan 1993; MZUSP 54334, 2, 93.4 – 120.6 mm SL, Rio Pium, Natal, 20 km north of Natal, F. Münz, 7 Oct 1998; **Alagoas:** MZUSP 9418, 3, 167.6 mm SL, Lagoa de Jiquiá, Mangabeira, city of São Miguel, E.Dente, 195?; **Sergipe:** MZFS 16162, 6, 105.1 – 114.6 mm SL, Rio São Francisco, Ilha das Flores; MZFS 16163, 5, 102.7 – 123.2 mm SL, Rio São Francisco, Ilha das Flores; **Bahia:** MZFS 6204, 1, 111.6 mm SL, Berlinque beach, Ilha de Itaparica, L. E. Moraes, P. R. D. Lopes & J. T. Oliveira, 27 June 2007; MZFS 10145, 7, 95.5 – 148.8 mm SL, tributary of Rio Cachoeira Grande, 16 June 2007; MZUSP 1574, 4, 110.5 – 125.9 mm SL, Ilha de Itaparica, C.E.Dawson et al., 25 Aug 1972; MZUSP 39056.0, 1, 146.2 mm SL, Rio Caí, Fazenda Limoeiro close to Cumuruxatiba, Expedição MZUSP/USNM, 20 Mar 1985; MZUSP 102662, 4, 97.0 – 131.2 mm SL, Mangrove, Ilhéus, 14º37'32"S 039º04'08"W, 13 Feb 2009; MZUSP 112640, 19, 99.2 – 146.3 mm SL, Rio Mucurizinho, Mucuri, on the road between BR 101 and Mucuri, just before arriving in the city, Rio Mucuri basin, 18.06453º S 39.56335º W, 8 Aug 2012; MZUSP 112464, 2, 120.9 – 140.0 mm SL, Rio Pitanga, Valença, east drainage, near the Cantagalo waterfall, Rio Una basin, 13º26'35.12" S 39º06'42.26" W, 12 Aug 2012; UFBA 03661, 4, 83.6 – 117.4 mm SL, Rio Bu, drainage of Rio Inhambupe, under a bridge on Linha Verde, A. Zanata, M. Geraldes & P. Camelier, 29 May 2005; UFBA 03662, 1, 134.8 mm SL, tributary of Rio Inhambupe in Bahia, 400 to 500 m from the Linha Verde, A. Zanata, M. Geraldes & P. Camelier, 29 May 2005; UFBA 07669, 1, 106.9 mm SL, coast of Bahia, Ilhéus, G. Carvalho, 13 Sept 2011; ZUEC 2990-2994, 5, 124.9 – 177.0 mm SL, estuary of Rio Mucuri, city of Mucuri, J. L. Gasparini, H. S. Sá & C. Zamprogno, 7 July 1993; **Espírito Santo:** MZUSP 27588, 1, 157.2 mm SL, Rio Conceição on BR-101, close to Guarapari, Expedição MZUSP/USNM,13 Nov 1982; MZUSP 90777, 1, 101.3 mm SL, Rio Iconha, Iconha, 20°49'13"S 40°47'46"W, 15 Apr 2006; **Rio de Janeiro:** MZUSP 9410.0, 11, 102.2 – 137.4 mm SL, Atafona, N. A. Menezes & J. Baskin, 7 Aug 1963; MZUSP 9411.0, 15, 92.9 – 114.1 mm SL, São João da Barra, Rio Paraíba, N. A. Menezes & J. Baskin, 26 July 1963; MZUSP 9412.0, 14, 94.1 – 152.4 mm SL, Atafona, Ilha do Peçanha, N. A. Menezes,19 Apr 1964; MZUSP 64508.0, 1, 129.3 mm SL, Atafona, Expedição do Departamento de Zoologia,19 Mar – 9 Apr 1964; MZUSP 64510.0, 3, 94.8 – 141.2 mm SL, Atafona, Ilha da Convivência, Expedição do Departamento de Zoologia, 19 Mar 1964; MZUSP 64623.0, 1, specimen with a broken tail (i.e., not measured), Rio Paraíba do Sul, São João da Barra, Expedição do Departamento de Zoologia,

19 Mar – 9 Apr 1964; MZUSP 64626, 6, 109.7 – 146.8 cm SL, Pontal (lagoons), Atafona, Expedição do Departamento de Zoologia,19 Mar 1964; **São Paulo:** MZUSP 859, 2, specimens with broken tails (i.e., not measured), Cubatão, Bicego, 1897; MZUSP 37373, 3, 101.4 – 138.9 mm SL, Rio Cabucu, Baixada Santista, H. A. Britski, S. Kullander, M. Ortega & M. Villacorta, 21 Aug 1980; MZUSP 58735.0, 1, 144.1 mm SL, Rio Tetequera, tributary of Rio Guaraú, Peruíbe, 24°23'9"S 47°3'56"W, C. P. Silva & N. Silva, Mar 1997; MZUSP 103644, 1, 93.7 mm SL, marginal streams of Jurubatuba River, tributary of Santos estuary, Santos, G. Mattox & J. M. P. Iglesias, 3 Oct 2007; MZUSP 115451, 3, 109.1 – 131.0 mm SL, stream on the SP-61 highway at kilometer 17 before the Iporanga condominium towards Guarujá-Bertioga, Guarujá, 23.890484°S 46.173422°W, B. Abreu-Santos & J. A. P. Santos, 6 Mar 2012; MZUSP 125711, 18, 88.4 – 150.0 mm SL, Rio Prumirim, Ubatuba, G. M. T. Mattox, Apr 2015; ZUEC PIS 2299-2306, 10, 109.3 – 142.5 mm SL, Rio Itamambuca, Ubatuba, 23°24'S 45°00'W, W. R. Silva, 6 May 1978; ZUEC 5064, 2, 86.0 – 107.3 mm SL, Rio Mococa, Caraguatatuba, A. C. Filho, 10 Sept 2000; ZUEC 5141, 7, 124.4 – 161.9 mm SL, Rio Itamambuca, Ubatuba, I. Sazima, W. Uieda & V. S. Uieda, 25 Oct 1979; ZUEC 5392, 2, 108.6 – 124.1 mm SL, Rio da Fazenda, Ubatuba, I. Sazima,11 Jan 2001; ZUEC PIS 5410, 4, 113.7 – 139.9 mm SL, Rio Escuro, Ubatuba, 23°29"S 45°09"W, I Sazima et al., 25 Mar 1983; ZUEC 5494, 1, 126.2 mm SL, Rio Escuro, Ubatuba, I. Sazima, 28 Sept 2001; ZUEC PIS 9000, 3, 94.1 – 113.9 mm SL, Córrego da Paciência, Praia da Fazenda, Ubatuba, Parque Estadual da Serra do Mar, Picinguaba nucleus, 23°21'22"S 44°51'51"W, I. Sazima et al., 28 July 2001; ZUEC PIS 9055, 2, 113.4 – 116.7 mm SL, Córrego da Paciência, Praia da Fazenda, Ubatuba, Parque Estadual da Serra do Mar, Picinguaba nucleus, 23°21'22"S 44°51'51"W, I.Sazima et al., 8 Nov 2001; ZUEC 3004, 9, 98.8 – 128.6 mm SL, mangrove, Praia das Palmas, Ilha Anchieta, Ubatuba, I. Sazima, R. L. Moura & C. Sazima, 7 Sept 1996; ZUEC PIS 9572, 2, 96.6 – 109.7 mm SL, Rio Tabatinga, Praia de Tabatinga, Caraguatatuba, 23°34'40"S 45°16'32"W, A. Carvalho-Filho, 13 Oct 2000; ZUEC PIS 4754, 2, 86.6 – 96.6 mm SL, Ubatuba, Saco da Ribeira, 23°30'S 45°07'W, I. Sazima, 22 Feb 1970; **Rio Grande do Sul:** UFRGS 3646, 1, 102.2 mm SL, Tramandai pond, Tramandai,11 Apr 1984; UFRGS 3647, 1, specimen with a broken tail (i.e., not measured), Tramandai pond, Tramandai, 11 Apr 1984.

2.8.Tables list

Tab. 1. Frequency distribution of the number of dorsal-fin rays of *Microphis lineatus* in each Brazilian state analysed.

Tab. 2. Frequency distribution of the total number of subdorsal rings of *Microphis lineatus* in each Brazilian state analysed.

Tab. 3. Frequency distribution of the number of tail rings of *Microphis lineatus* in each Brazilian state analysed.

Tab. 4. Frequency distribution of the number of trunk rings of *Microphis lineatus* in each Brazilian state analysed.

Tab. 5. Frequency distribution of the number of brood pouch rings of *Microphis lineatus* in each Brazilian state analysed.

Tab. 6. Frequency distribution of the total number of body rings of *Microphis lineatus* in each Brazilian state analysed.

Tab. 7. Frequency distribution of the point of origin of dorsal fin of *Microphis lineatus* in each Brazilian state analysed.

Tab. 8. Frequency distribution of the number of the left pectoral-fin rays of *Microphis lineatus* in each Brazilian state analysed.

Tab. 9. Frequency distribution of the number of the right pectoral-fin rays of *Microphis lineatus* in each Brazilian state analysed.

Tab. 10. Frequency distribution of the number of caudal-fin rays of *Microphis lineatus* in each Brazilian state analysed.

Tab. 11. Frequency distribution of the number of anal-fin rays of *Microphis lineatus* in each Brazilian state analysed.

Tab. 12. Frequency distribution of the number of longitudinal rows of eggs of *Microphis lineatus* in each Brazilian state analysed.

Tab. 13. Morphometric data of *Microphis lineatus* obtained. Subunits of the body are presented in relation to the standard length, and subunits of the head are presented in relation to the length of the head. Number refers to the total number of specimens analysed.

Tab. 14. Number of rays from the left (NPRL) and right (NPRR) pectoral fins of *Microphis lineatus*.

2.9.Figures list

Fig. 1. Specimen of *Microphis lineatus* from the lot: ZUEC 5141, 148.7 mm SL, Rio Itamambuca, Ubatuba, SP.

Fig. 2. Distribution of *Microphis lineatus* along the Brazilian coast based on 49 analysed lots.

Fig. 3. Number of *Microphis lineatus* specimens analysed by Brazilian state (n = 209).

Fig. 4. Number of male (M) and female/juvenile (F/J) specimens of *Microphis lineatus* in relation to the total number of specimens (n = 209).

Fig. 5. Positive correlation between the dissimilarity of the *Microphis lineatus* meristic data counts, in the y-axis – number of dorsal-fin rays (NDR), total number of subdorsal rings (TNSR) and number of tail rings (NLR) – and the variation in the distance between the analysed lots collection points, represented by the latitude (°), in the x-axis, and by the geographical distance between the points coordinates (km), represented by the color gradient of the plotted points resulting from the data permutation.

2.10.Supplementary files list

S1. List of the Study Material. Lots were organized by the order of Brazilian coastal states in the north-south direction and include: collection acronym and catalog number, number of specimens examined, standard length range (mm SL), collection site, collectors and date of collection were provided for each lot.

2.11. Tables and figures

Tab. 1. Frequency distribution of the number of dorsal-fin rays of *Microphis lineatus* in each Brazilian state analysed.

	Number of dorsal-fin rays											
State	42	43	44	45	46	47	48	49	50	51	52	Total
Amapá		1		1			1					3
Pará	1				1							2
Rio Grande do Norte		3	1		7	1	1	1				14
Alagoas						1		2				3
Sergipe					3	1	3	1	1	2		11
Bahia		1	2	7	9	9	6	8	3	2	2	49
Espírito Santo							1		1			2
Rio de Janeiro					3	5	9	15	6	9	4	51
São Paulo				1	5	10	12	15	13	12	3	71
Rio Grande do Sul										1	1	2

Tab. 2. Frequency distribution of the total number of subdorsal rings of *Microphis lineatus* in each Brazilian state analysed.

	Total number of subdorsal rings												
State	8.25	8.50	8.75	9	9.25	9.50	9.75	10	10.25	10.50	10.75	11	Total
Amapá	1		2										3
Pará		2											2
Rio Grande do Norte		2	3	4	2	2	1						14
Alagoas				2		1							3
Sergipe		2	1	2		3	3						11
Bahia		2	3	10	6	8	5	10	3	1	1		49
Espírito Santo						1		1					2
Rio de Janeiro					6	8	16	9	7	5			51
São Paulo				1	5	19	24	13	8			1	71
Rio Grande do Sul						1		1					2

Tab. 3. Frequency distribution of the number of tail rings of *Microphis lineatus* in each Brazilian state analysed.

State	Number of tail rings										
	18	19	20	21	22	23	24	25	26	27	Total
Amapá					2		1				3
Pará					2						2
Rio Grande do Norte					1		2	10			13
Alagoas								1			1
Sergipe								6	4		10
Bahia	1	1	1	1		1	12	24	3		44
Espírito Santo							1	1			2
Rio de Janeiro			1	1	1		3	27	9	1	43
São Paulo					1	5	14	41	3		64
Rio Grande do Sul									1		1

Tab. 4. Frequency distribution of the number of trunk rings of *Microphis lineatus* in each Brazilian state analysed.

State	Number of trunk rings			
	19	20	21	Total
Amapá	3			3
Pará	2			2
Rio Grande do Norte	1	10	3	14
Alagoas		1	2	3
Sergipe	1	10		11
Bahia	1	38	10	49
Espírito Santo	1	1		2
Rio de Janeiro		45	6	51
São Paulo	2	64	6	72
Rio Grande do Sul		2		2

Tab. 5. Frequency distribution of the number of brood pouch rings of *Microphis lineatus* in each Brazilian state analysed.

	Number of brood pouch rings					
State	19	20	21	22	23	Total
Amapá						0
Pará		1				1
Rio Grande do Norte		2	1	1		4
Alagoas					2	2
Sergipe		1	2	1		4
Bahia		5	4	1	3	13
Espírito Santo					1	1
Rio de Janeiro	1	1	3	3	3	11
São Paulo	2	6	12	3	1	24
Rio Grande do Sul						0

Tab. 6. Frequency distribution of the total number of body rings of *Microphis lineatus* in each Brazilian state analysed.

	Total number of body rings									
State	39	40	41	42	43	44	45	46	47	Total
Amapá			2		1					3
Pará			2							2
Rio Grande do Norte				1		2	9	1		13
Alagoas							1			1
Sergipe						1	5	4		10
Bahia	2	1	1		1	7	26	6		44
Espírito Santo					1		1			2
Rio de Janeiro			2		1	3	25	11	1	43
São Paulo				1	4	15	38	6		64
Rio Grande do Sul								1		1

Tab. 7. Frequency distribution of the point of origin of dorsal fin of *Microphis lineatus* in each Brazilian state analysed.

State	Point of origin of dorsal fin								
	2.25	2.50	2.75	3.00	3.25	3.50	3.75	4.00	Total
Amapá				1		1	1		3
Pará					1	1			2
Rio Grande do Norte		1	5	5	1	1	1		14
Alagoas		1		1		1			3
Sergipe		5	4	2					11
Bahia	3	3	6	25	6	4	1	1	49
Espírito Santo					1	1			2
Rio de Janeiro		4	4	20	14	6	1	2	51
São Paulo		4	5	31	15	12	2	3	72
Rio Grande do Sul			1				1		2

Tab. 8. Frequency distribution of the number of the left pectoral-fin rays of *Microphis lineatus* in each Brazilian state analysed.

State	Number of pectoral-fin rays (left)					
	17	18	19	20	21	Total
Amapá	2			1		3
Pará		1	1			2
Rio Grande do Norte		1	7	5	1	14
Alagoas				3		3
Sergipe		1	5	4	1	11
Bahia		2	20	22	5	49
Espírito Santo			1	1		2
Rio de Janeiro		3	17	26	4	50
São Paulo		6	25	37	4	72
Rio Grande do Sul			1	1		2

Tab. 9. Frequency distribution of the number of the right pectoral-fin rays of *Microphis lineatus* in each Brazilian state analysed.

	Number of pectoral-fin rays (right)						
State	17	18	19	20	21	22	Total
Amapá		2		1			3
Pará	1		1				2
Rio Grande do Norte			6	6	2		14
Alagoas			2	1			3
Sergipe			5	5		1	11
Bahia		4	16	23	6		49
Espírito Santo			1		1		2
Rio de Janeiro			19	28	4		51
São Paulo		9	21	37	5		72
Rio Grande do Sul				2			2

Tab. 10. Frequency distribution of the number of caudal-fin rays of *Microphis lineatus* in each Brazilian state analysed.

	Number of caudal-fin rays				
State	8	9	10	11	Total
Amapá		3			3
Pará		2			2
Rio Grande do Norte	1	12			13
Alagoas		1			1
Sergipe		10			10
Bahia	1	43			44
Espírito Santo		2			2
Rio de Janeiro	1	39	1	2	43
São Paulo		64			64
Rio Grande do Sul		1			1

Tab. 11. Frequency distribution of the number of anal-fin rays of *Microphis lineatus* in each Brazilian state analysed.

State	Number of anal-fin rays				
	3	4	5	6	Total
Amapá		3			3
Pará		1	1		2
Rio Grande do Norte	1	12	1		14
Alagoas		2	1		3
Sergipe		11			11
Bahia	1	42	3		46
Espírito Santo		2			2
Rio de Janeiro		50		1	51
São Paulo		63	8		71
Rio Grande do Sul		2			2

Tab. 12. Frequency distribution of the number of longitudinal rows of eggs of *Microphis lineatus* in each Brazilian state analysed.

State	Number of longitudinal rows of eggs										
	3	4	5	6	7	8	9	10	11	12	Total
Amapá											0
Pará						1					1
Rio Grande do Norte			1					1			2
Alagoas				1						1	2
Sergipe		2		2							4
Bahia		1	1	2		2	1	1			8
Espírito Santo						1					1
Rio de Janeiro				2	1	2		1		1	7
São Paulo	1	2		6		2					11
Rio Grande do Sul											0

Tab. 13. Morphometric data of *Microphis lineatus*. Subunits of the body are presented in relation to the standard length, and subunits of the head are presented in relation to head length. Number refers to the total number of specimens analysed.

Character	Number	Range	Mean	Standard deviation
Standard length (SL)	183	76.8 - 177.0	118.6	–
Snout length (SL)	209	54.5 - 64.9	58.7	1.9
Snout depth (SD)	209	5.9 - 11.6	8	1.2
Snout width (SW)	209	4.0 - 9.4	7.1	1.1
Orbital diameter (OD)	209	9.4 - 15.9	12.8	1.1
Post-orbital length (POL)	209	25.3 - 32.1	28.6	1.2
Head depth (HD)	209	15.2 - 21.2	17.5	1.1
Pectoral-fin length (PFL)	183	1.6 - 2.8	2.3	0.2
Pectoral-fin base length (PBL)	183	1.7 - 2.6	2.2	0.1
Dorsal-fin base length (DBL)	183	11.5 - 18.8	15.6	1.2
Anal ring depth (AD)	183	1.7 - 3.9	3	0.3
Trunk depth (TD)	183	2.6 - 5.4	3.7	0.7
Body depth (BD)	183	2.5 - 4.5	3.3	0.4
Body width (BW)	183	2.2 - 3.4	2.7	0.2
Tail length (TL)	183	36.8 - 46.8	42.5	1.8
Brood pouch length (BPL)	51	33.1 - 45.9	39.2	2.8
Head length (HL)	183	17.0 - 21.7	19.3	0.8

Tab. 14. Number of rays from the left (NPRL) and right (NPRR) pectoral fins of *Microphis lineatus*.

	NPRL	NPRR
Mode	20	20
Range	17 - 21	17 - 22
Mean	19.54	19.60
Standard deviation	0.77	0.79
N sample	208	209

Fig. 1. Specimen of *Microphis lineatus* from the lot: ZUEC 5141, 148.7 mm SL, Rio Itamambuca, Ubatuba, SP. Scale bar = 10 mm.

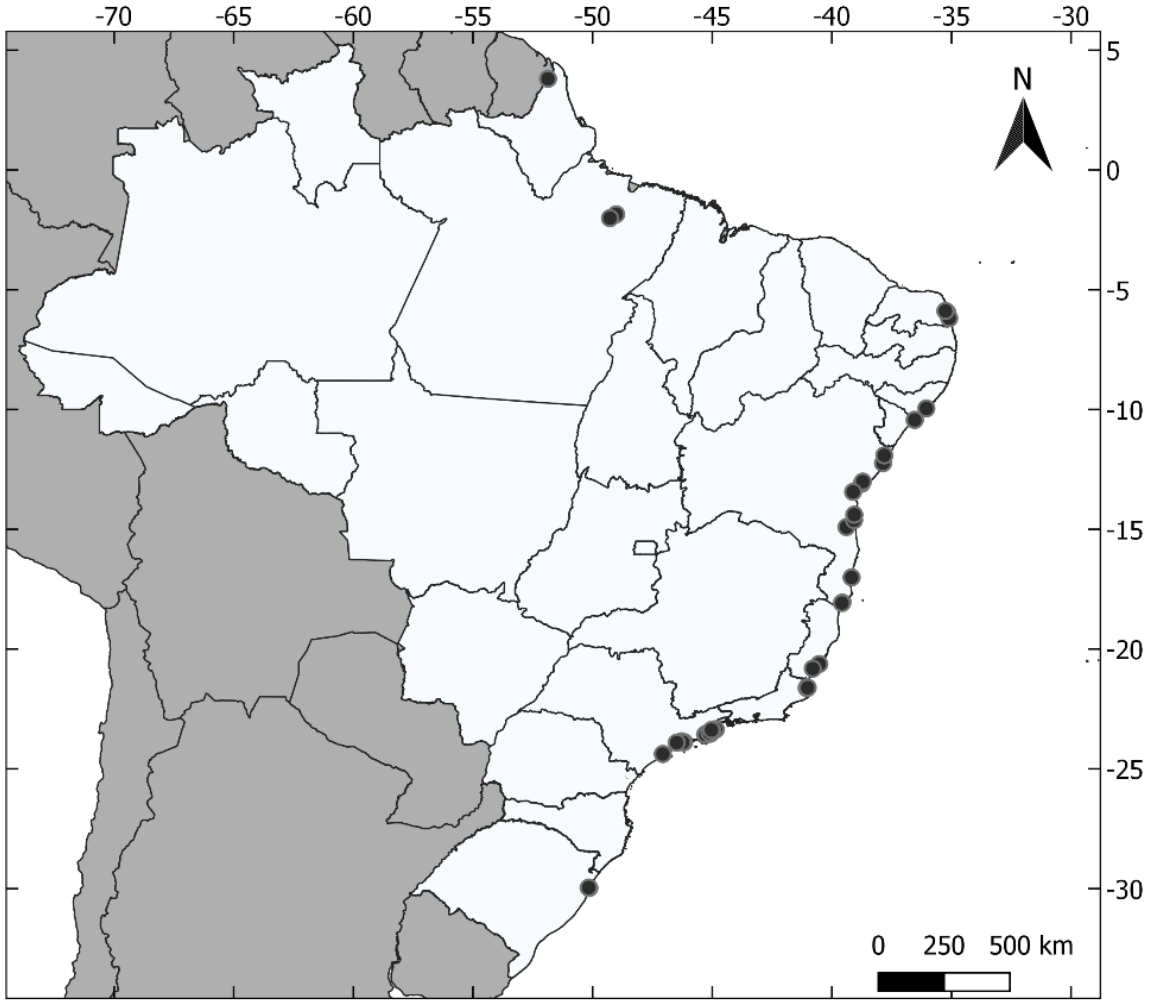


Fig. 2. Distribution of *Microphis lineatus* along the Brazilian coast based on 49 analysed lots.

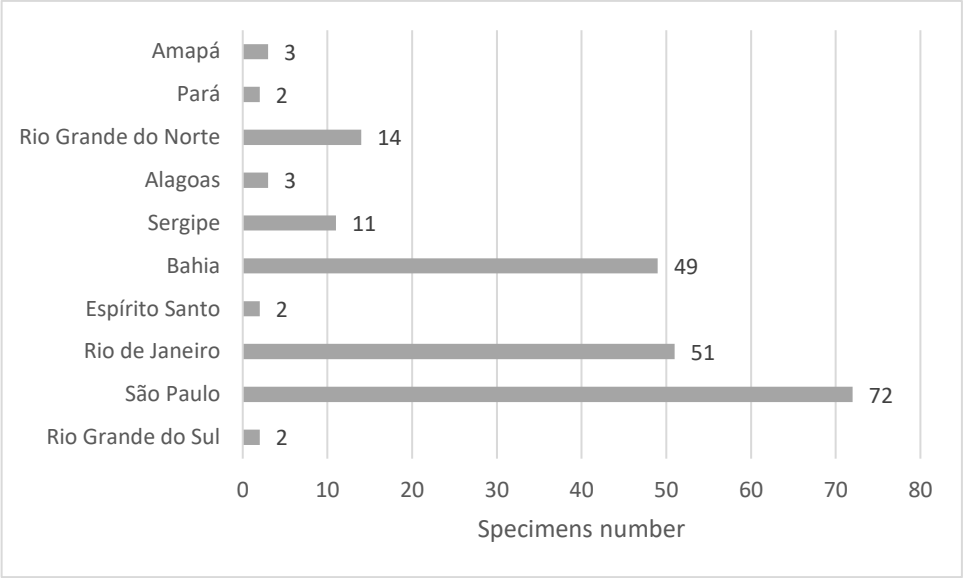


Fig. 3. Number of *Microphis lineatus* specimens analysed by Brazilian state (n = 209).

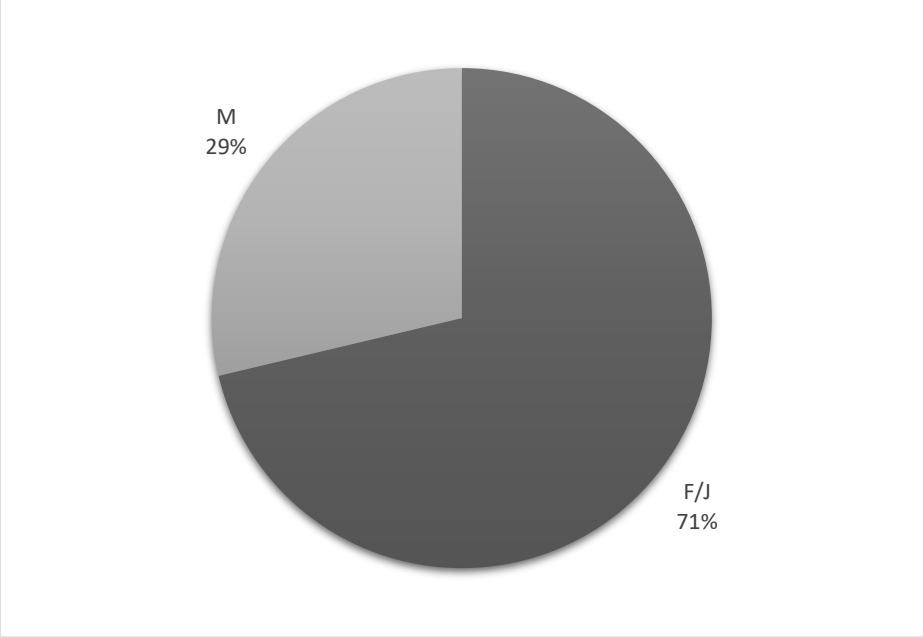


Fig. 4. Number of male (M) and female/juvenile (F/J) specimens of *Microphis lineatus* in relation to the total number of specimens (n = 209).

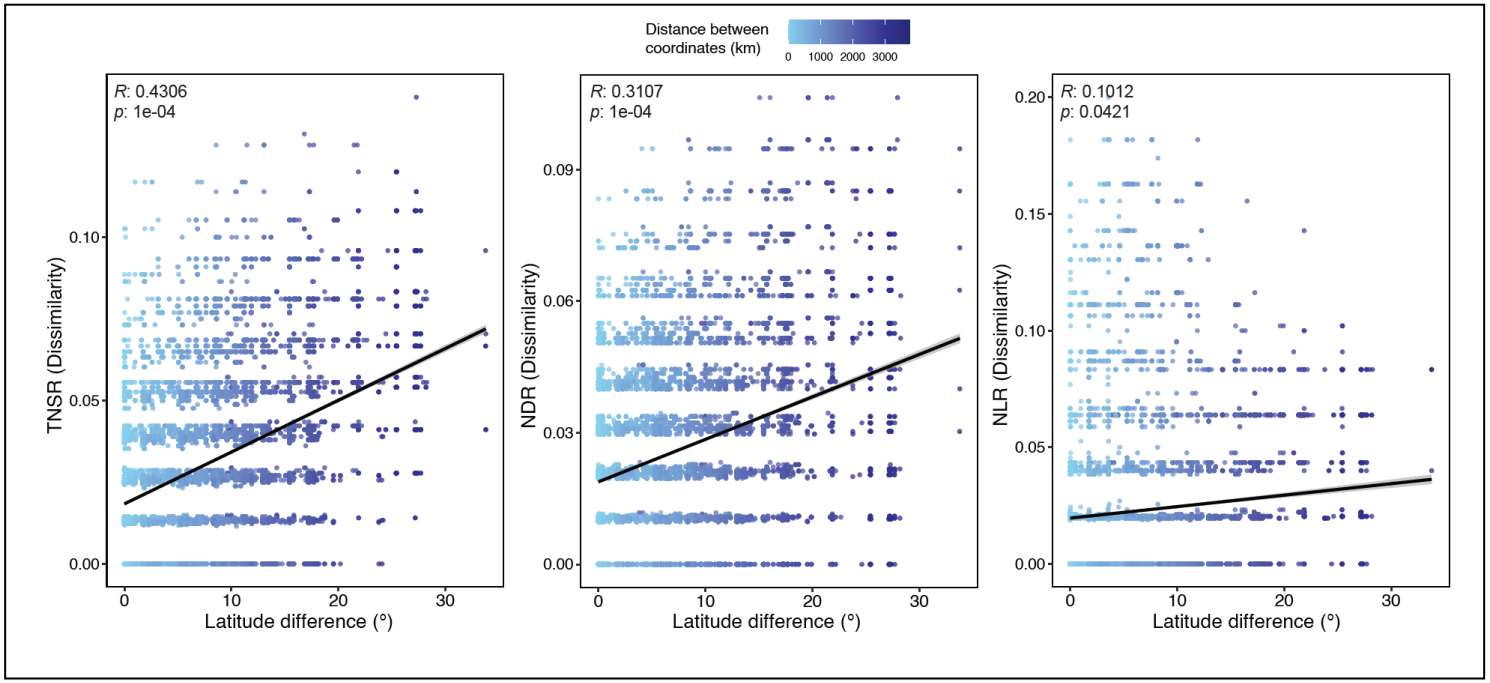


Fig. 5. Positive correlation between the dissimilarity of the *Microphis lineatus* meristic data counts, in the y-axis – number of dorsal-fin rays (NDR), total number of subdorsal rings (TNSR) and number of tail rings (NLR) – and the variation in the distance between the analysed lots collection points, represented by the latitude (°), in the x-axis, and by the geographical distance between the points coordinates (km), represented by the color gradient of the plotted points resulting from the data permutation.

ANEXO A – REGRAS DE FORMATAÇÃO DA REVISTA NEOTROPICAL ICHTHYOLOGY

Neotropical Ichthyology

INSTRUCTIONS TO AUTHORS

(May, 2020)

Scope and policy

Neotropical Ichthyology prioritizes articles on native species in their original basins. Articles focusing on aquaculture should be submitted to journals focusing on Animal Science.
Submitted manuscripts must report on original research, provide clear theoretical foundations, describe the objectives and/or hypotheses under consideration, and employ appropriate sampling and analytical . Descriptive studies which have a clear conservation or management focus and/or demonstrate relevance to a broad audience by addressing interesting scientific questions can be eventually published only when they (i) focus on important species lacking critical information about their biology and natural history; (ii) investigate species occurring in highly disturbed environments, especially those impacted by mining, large hydroelectric plants and urban areas, with an explicit conservation or management problem; or (iii) investigate species in a threat category or the DD (Data Deficient) category on red lists. Ultimately, the decision to consider a descriptive study is a prerogative of the editors.
Casual observations, scientific notes or descriptive studies not associated with relevant theoretical issues will not be considered.

Submission of manuscripts

Submissions to **Neotropical Ichthyology** occur exclusively online through the ScholarOne portal. Manuscripts must be submitted as digital files at http://mc04.manuscriptcentral.com/ni-scielo. The Editor-in-Chief will screen each manuscript submitted to **Neotropical Ichthyology** to verify whether it is within the journal's scope, presents original research and follows the instructions of the journal to authors. After passing through the initial screening, articles are assigned to a Section Editor, who then assigns an Associate Editor to start the single blind review process. The journal is open for submissions to all researchers on Neotropical ichthyofauna.

With each new *manuscript* submission, *authors* must include a **cover letter** stating that the article constitutes original research and is not being submitted to other journals. The letter should also explain to editors why your article should be published, highlight the strengths of your research and outline the recommendations that can be drawn from your work. In multi-authored papers, the author responsible for

submission must declare in the cover letter that all coauthors are aware of and agree with the submission. To that end, please ensure that all coauthors have read and approved the final version.

Before starting the submission, the corresponding author must obtain the validated ORCIDS from all authors and co-authors and ask them to update their profiles in the system BEFORE submission. Authors may register for an ORCID at http://orcid.org/, and provide ORCID numbers for all coauthors during submission. The mailing addresses and valid e-mail addresses for all authors must be entered in the appropriate forms during manuscript submission as well. Do not translate institution names. During the submission, indicate at least five possible reviewers, providing name, institution, country, and valid e-mail addresses. You may also indicate your opposition to particular reviewers or conflicts of interests, if applicable.

Please, read carefully and follow all applicable rules prior to submission. Manuscripts that do not meet the journal formatting requirements, lack required files, or are written in poor English will be returned to authors without review.

Costs

Paper charges will be billed to the corresponding author with costs of R$400 reais for article. For authors from outside Brazil the values will be converted to US dollars based on the current exchange rate.

All charges are waived when one or more authors are current SBI members (having paid the membership fee in the current year) or when the paper has been invited through the system. Contact the SBI Secretary (http://www.sbi.bio.br/en/membership) for membership rates and payment procedures.

Form and preparation of manuscripts

General form and preparation of manuscripts

Do not duplicate information among the text, figures and tables. Submit only figures and tables that are strictly necessary. Supplementary files such as appendices, and videos should be uploaded already formatted, as pdf or video files in MP4 format.

For taxonomic papers, please also refer to: ***Neotropical Ichthyology* taxonomic style below**.

Licensing

Since 2015, Neotropical Ichthyology publishes under a Creative Common BY license (Attribution). Articles accepted for publication become property of the journal.

Copyright

Though authors retain the copyright of their articles, by submitting manuscripts to Neotropical Ichthyology, they grant the journal a license to publish the article

electronically, to identify itself as the original publisher, and to distribute and promote the article on the journal's website and via social media.

Ethical statement

As recommended by the Brazilian Council of Animal Experimentation Control, we ask you to provide the protocol numbers authorizing the research by the Animal Ethics Committees of your institution and the permit numbers or collection licenses authorizing field research.

E.g.: Experiments were approved by the Ethical Committee for Animal Use in Experiments of the Instituto Nacional de Pesquisas da Amazônia (CEUA number ###/year ###) and Collection Licenses of SISBIO number ####.

If your work did not require such permissions, state this explicitly. For manuscripts from countries other than Brazil, please provide the documentation required in the respective country.

Science Communication

NI has now a Science Communication Editor, which will be in charge of preparing press releases, social media posts and other promotional material for selected articles. If you prefer to produce such material yourself, please produce materials such as a cartoon, text, video, infographic or any other material during the peer-review period. It will be asked to you, in case of acceptance of your article, to be displayed on NI's website or other medias. Otherwise, I would like you authorize the journal to do it for you, if pertinent.

Author contributions and competing interests

This information is required at the time of article submission. Ensure that all authors and co-authors are aware and agree, as they will be included in the publication.

FORMATTING RULES

Please, be sure you have carefully read all the items below

FILE AND PAGE SETUP

Manuscript files must be in the DOC, DOCX or RTF formats. Do not lock or protect the file. Formats such as XLS, XLSX or PDF will NOT be accepted.

The document file cannot include headers, footers, or footnotes (except page number). Do not format text in multiple columns. Although no page limit is imposed, manuscripts should always be as concise as possible.

Text should be aligned to the left (except if otherwise mentioned), not fully justified, not indented by tab or space and not underlined. Do not hyphenate words at line breaks (though hyphens can be used in compound constructions, such as dorsal-fin rays, as appropriate).

All text must be Times New Roman font size 12, with 1.5 line spacing. Do not number lines. The font "symbol" can be used to represent the following characters: χ μ θ ω ε ρ τ Ψ υ ι ο π α σ δ Φ γ η φ κ λ ∋ ϖ β ν ≅ Θ Ω Σ Δ Φ. Spell

out numbers from one to nine, except those that refer to numerical values, scale counts, and when referencing figures and tables. Also, spell out numbers that begin a sentence.

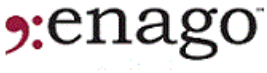
Abbreviations used in the text must be listed under Material and Methods; except for those in common use (***e.g.***, min, km, mm, kg, m, sec, h, ml, L, g). For measurements, use the metric system.

LANGUAGE

Text must be submitted in English. Avoid clichés, slang, and colloquial words or expressions such as "In the present study". If none of the authors are native English speakers, we recommend that you contract with a professional language editing and copyediting services or have the manuscript read by a native English speaking colleague prior to submission. Authors are free to choose any certified service, but Neotropical Ichthyology authors receive a discount from these two companies.

AMERICAN JOURNAL EXPERTS®

(10% discount)



(CODE: PESQUISA, 20% discount)

MANUSCRIPT FILE

TITLE

New taxa names should not appear in the title or abstract. Center the title and present it in boldface, without quotation marks, with sentence-style capitalization, and with subordinate taxa separated by ":". Titles must reflect the contents of the paper and use scientific names rather than vernacular names. Do not provide taxonomic authorship in the title. ***E.g.:***

A new species of loricariid catfish from the rio Ribeira de Iguape basin, Brazil (Ostariophysi: Siluriformes)

AUTHORS

As the submitting author will be responsible for completing information at submission, it is mandatory that all authors have reviewed, discussed, and agreed with the contents of the manuscript and the order of authorship prior to submission. All co-authors must have contributed substantially to all article steps. Capitalize only the initial letters of authors' names. Do not abbreviate first name of authors and separate the names of the last two authors by "and". We encourage presenting the full middle names of the authors, except when the number of authors is more than four. In case of authors from different institutions, use superscript numerals to identify each one in regular font (not italics). Superscript numerals can also be used to identify multiple addresses for each individual author. For Hispanic surnames, insert a hyphen between the paternal and the maternal surname if the author wishes to be cited with both. ***E.g.:***

Heraldo Antônio Britski1, Naércio Aquino de Menezes1, Hernán Ortega2 and John Lundberg3

AUTHORS ADDRESSES

Full mailing addresses and a valid email of all authors must be provided, including institution name, ZIP codes, cities (no comma between ZIP and city), states and countries. For Brazilian and American states, use standard abbreviations preceded

by comma, and always present the country name in English. Footnotes should not be used. List emails as part of the institutional address. When there is more than one author at a given institution, insert initials of each author name before their respective email address. Provide ORCIDs of all the authors and coauthors in the main text and of the submission author in the system. If any author is not registered yet, one must register in http://orcid.org/. Indicate the corresponding author by adding (corresponding author) after the ORCID. Do not use period.
E.g.:
[1] Seção de Peixes, Museu de Zoologia da Universidade de São Paulo, Av. Nazaré, 481, Ipiranga, 04263-000 São Paulo, SP, Brazil. (HAB) heraldo@usp.br, ORCID http://orcid.org/0000-0002-5593-9651 (corresponding author), (NAM) naercio@usp.br, ORCID https://orcid.org/0000-0002-9634-6051
[2]Departamento de Ictiología, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Av. Arenales 1256, Apartado, 14-0434 Lima, Peru. hortega.musm@gmail.com, ORCID http://orcid.org/0000-0002-4396-2598
[3]Department of Ichthyology, The Academy of Natural Sciences of Drexel University, 1900 Benjamin Franklin Parkway, 19103-1195 Philadelphia, PA, USA. mhs58@drexel.edu, ORCID https://orcid.org/0000-0002-5671-9933

TEXT

The body of text may employ named heading and subheadings, which cannot be lettered or numbered. All sections are left justified, except the primary headings, which should appear centered in small caps and bold font. Employ the following heading, in the cited order:

Abstract

Abstracts must appear as a single paragraph with fewer than 200 words in English. Do not include new taxa names, authorship or references. Do not indent. Remember that this is the first piece of your article that will be viewed by each potential reader. Include information showing the importance and relevance of your article to encourage the reader to read your entire paper.

Resumo or **Resumen**

Provide a concise (maximum 200 words) and accurate Portuguese or Spanish translation of the English abstract.

Keywords, Palavras-chave ou Palabras clave

Provide up to five capitalized keywords in English, in alphabetic order and separated by commas. Do not use words already contained in the title, nor Neotropical (which appears in the name of the journal). If the article provides an identification key, include that as keyword in the English and translated lists. According to the language you provide the Resumo or Resumen, choose present Palavras-chave or Palabras clave. The order of them also is arranged alphabetically, and then the sequence of the words might differ from those in English. ***E.g.***:

Keywords: Conservation, Identification key, Ostariophysi, Taxonomy
Palavras-chave: Chave de identificação, Conservação, Ostariophysi, Taxonomia
OR
Palabras clave: Clave de identificación, Conservación, Ostariophysi, Taxonomía

Running Head

Provide a suggested running head of up to 50 characters. It must concisely reflect the content of the article. Do not include vernacular names or species authorship here. ***E.g.***:

New *Astyanax* species from the rio Ribeira de Iguape

Introduction

Provide taxonomic authorship in the first appearance of species names in the text. See Nomenclature Section below for further instructions.

Material and Methods

If two heading levels are used, follow this format:

Sampling sites. Collections were carried out in…
Statistical analyses. Data were analyzed…
In case of listing Examined Material, provide a list of institutional acronyms in Material and Methods section OR a reference to a published paper with a list of acronyms in Material and Methods. Also, reference(s) for species identification and classification used must be provided.

Results

Do not unite Results and Discussion as a unique section.

Discussion

Do not provide a separate Conclusion section. However, we encourage highlighting conclusions as the last paragraph(s) of the Discussion.

Acknowledgments

Acknowledgments are optional but encouraged. If included, they must be concise and include both first and last names of persons. If you wish to provide the institutions of people thanked, use abbreviate names for institutions, which the full name has been provided in the Material and Methods. Names of sponsor institutions should be listed in their original spelling and not translated to English. Collections permit numbers and approvals of ethics committees can be listed here OR in the Material and Methods section.

References

See detailed rules bellow.

VOUCHER SPECIMENS
Voucher specimens of all species examined must be deposited in a recognized scientific research collection, even in studies focusing on a single well-known species. A list of catalog numbers of voucher specimen(s) must be furnished in all manuscripts.

GENETIC SEQUENCES
Authors must deposit genetic sequence data used for phylogenetic or other analyses in a public online depositary, and include a Table or Appendix in their manuscript with the following information: museum acronym and catalog number; online depository name (*e.g.* BOLD, GenBank); depository accession number (it is appropriate to use "pending" prior to acceptance of a manuscript, but following

acceptance of a manuscript, these numbers must be made available as a condition for final publication); the marker gene/locus (e.g. CO1, cytB, RAG2).

NOMENCLATURE

Species, genera, and Latin terms (*et al., in vitro, in vivo, vs., i.e, e.g.*) must be in italics. Cite scientific names according to the ICZN (http://iczn.org/iczn/index.jsp). Authorship should be given at the first reference to a species or genus. Spelling, valid names and authorship of species must be checked in the Catalog of Fishes at http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Latin terms presented between the generic and specific names (cf., aff., etc.) are not in italics (***e.g.,*** *Hoplias* aff. *malabaricus*).

The genus name must always be fully spelled at its first appearance, at the beginning of a sentence and at least once in each figure and table caption(s). After first mention, the first letter of the genus name followed by the full species name may be used (***e.g.,*** *H.* aff. *malabaricus*) as long as the abbreviation leaves no possibility of confusion with another generic name mentioned in the manuscript. In the case of possible confusion, the abbreviation can include more than the first letter to allow the differentiation of genera beginning with the same letter.

TABLES

Tables must be numbered sequentially in Arabic numerals according to the order of citation in the text and be cited in the text using the following formats: Tab. 1, Tabs. 1–2, Tabs. 1, 4. Approximate locations where tables should be inserted must be indicated in upper case, along the right margin of the text, as in:
TABLE 1

Note: Use an **n-dash** for ranges (to automatically create n-dash in Word type "something – something" *(number-space-hyphen-space-number))*.
In table captions, the word Tab., its respective number and final period after the number should be in bold (***e.g.,*** **Tab. 1.**...). End the caption in a period. Captions must be selfexplicative.
If genus names appear in a caption, spell out the name at least once.
Tables must be constructed in cells using lines and columns. Do not format tables with "tab" or "space". Tables should not contain visible vertical lines or footnotes [contents of footnotes must be included in the caption].
List all captions at the end of the manuscript, in the following format. ***E.g.:***

Tab. 1. Monthly variation of the gonadosomatic index in *Diapoma pyrrhopteryx* and *D. speculiferum*...

FIGURES

Figures cannot be submitted as images inserted in Word files. Figures must be submitted as high quality individual files. For b&w figures, they must be saved in TIFF format, gray scale, 8.5 or 17.5 cm width, 600 dpi. Color figures must be in TIFF format, CMYK, 8.5 or 17.5 cm width and 300 dpi.
Composed figures must fit either the page (17.5 cm) or column width (8.5 cm). Text included in graphs and pictures must have a font size compatible with reductions to page or column width.
Figures must be numbered sequentially in Arabic numerals according to their order of citation in the text. Cite figures in the text using the following formats: Fig. 1, Figs. 1–2, Fig. 1A, Figs. 1A–B, Figs. 1A, C. Indicate the approximate locations where figures should be inserted in upper case, along the right margin of the text, as in:
FIGURE 1

Note: Use an **n-dash** for ranges (to automatically create n-dash in Word type "something – something" *(number-space-hyphen-space-number))*.

In each figure caption, the word Fig., its respective number and period are in bold (***e.g.,*** **Fig. 1.**…). End each caption with a period. Captions must be self-explicative. If genus names appear in a caption, spell out the name at least once. Do not include symbols in the caption, but rather replace them with text (***e.g.,*** black triangle) or include a legend in the figure itself.
Indicate figure subsections in upper case and bold letters in both in the figure and caption. Do not use parentheses after letters. Cite figures from other articles using the same formats as figures published in the present article, but do not capitalize them.
Illustrations must include either a scale or reference to the size of the item in the figure caption. List all captions at the end of the manuscript, in the following format. ***E.g.***:

Fig. 1. Otoliths of representatives of Otophysi. **A**. *Brycon hilarii*; **B.** *B. orbignyanus*; **C.** *Pimelodus maculatus*; and **D.** *Sternopygus macrurus*. (Scale bars = 1 mm), lapillus (black triangle), asteriscus (white dot) and sagittal (red star), according to fig. 2 of Campana (2001).

SUPPLEMENTARY FILES
Upload appendices, videos, datasets and other complementary materials as supplementary files. Provide the files formatted as you wish it appear, but in some format that allow edition. Videos must be in MP4 format. Identify these files in the text by a bolded letter **S** followed by sequential numbers in Arabic numerals. Indicate in the text that those will appear only in the online version (***e.g.***… as shown in the video **S1**, available only in the online version,…). List all captions at the end of the manuscript. ***E.g.:***

S1. Video of variation of tides...
S2. Spreadsheet with catalog numbers of all voucher specimens collected in…

PERSONAL COMMUNICATION
Personal communications should be included in the text of your document – cited in text only and not included in your reference list. Provide the full name, first and family name in full, and initials of middle names when applicable, and year of the personal communication. ***E.g.:***

The sample site had scarce riparian vegetation (Carlos A. R. Silva, 2018, pers. comm.).

Note: It is recommended you get permission from the source/author of your personal communication.

REFERENCES

Ensure that all citations in the text and the References coincide before submitting a manuscript.

References must be cited in the following formats in the text: Eigenmann (1915, 1921) or (Eigenmann, 1915, 1921; Fowler, 1945, 1948; Carvalho, 2001) or Eigenmann, Norris (1918) or, for more than two coauthors, Eigenmann *et al*. (1910a,b), always in chronological order and then in alphabetical order in case of more than one author cited. Do not include undergraduate monographs, conference papers, abstracts or technical reports. Include Masters Thesis or Ph.D. dissertations only if extremely necessary. Do not format references with "tab" or

"space" and present references in rigorous alphabetical order. In case of authors with surnames with prepositions, in Portuguese do not include the preposition (***e.g.***, Carlos Alberto da Silva = Silva CA), in Spanish do not include "de" (***e.g.***, María de Rueda = Rueda M), but include "Del" (***e.g.***, Angel Del Río = Del Río A), except for authors who usually self cite differently. Ignore prepositions for the purpose of alphabetization, as in the following example:

E.g. of sequence De Carli F
Devincenzi GJ
Eigenmann CH
Maldonado-Ocampo J
De Pinna MCC
Del Río A
Rueda M
Silva CA

Note: In case of self-citations using a convention other than those exemplified, please cite using your usual convention and, in the cover letter, mention your intention to maintain and standardize that usage in al your self-citations in this and other journals.

For more than six authors you can use *et al.* from the seventh in all categories of references, or list all the authors. Choose one pattern and follow it consistently for all references.

For authors using reference management software, Vancouver is the style closest to NI's but the citations must be as described above. For users of the **Mendeley** manager, which is free, the Neotropical Ichthyology style for citations and references is available. To insert it in your Mendeley, login the software > view > citation styles > get more styles > paste the link https://csl.mendeley.com/styles/78754841/neotropical-ichthyology. For more information see: https://www.elsevier.com/solutions/mendeley/support.

Note: Do not forget to put the scientific names of references in italics in **Mendeley** before importing the reference. To do this, use the HTML tags at the beginning of the word and at the end. ***E.g.:***

Reproductive biology of two species of Mugil: M. curema and M.liza **=**
Reproductive biology of two species of *Mugil*: *M. curema* and *M. liza*.

Example formats are listed below.

Book

Baumgartner G, Pavanelli CS, Baumgartner D, Bifi AG, Debona T, Frana VA. Peixes do baixo rio Iguaçu. Maringá: EDUEM; 2012.

Edited book

Reis RE, Kullander SO, Ferraris CJ, Jr., editors. Check list of the freshwater fishes of South and Central America. Porto Alegre: Edipucrs; 2003.

Chapter in a book

Pires T, Ohara W. Gasteropelecidae. In: Queiroz L, Torrente-Vilara G, Ohara W, Pires T, Zuanon J, Doria C, editors. Peixes do rio Madeira. São Paulo: Dialeto Latin America Documentary; 2013. p.206–11.

Note: You must present only **two** digits for last page if the previous digits coincide with the previous digits of the first page, separated by an **n-dash** (to automatically create n-dash in Word type "something – something" *(number-space-hyphen-space-number))*.

Journal Articles

Journal titles may be abbreviated according to the style used in the sites: https://images.webofknowledge.com/images/help/WOS/R_abrvjt.html, http://cassi.cas.org/search.jsp, http://www.ncbi.nlm.nih.gov/nlmcatalog/journals, or others.
In case you do not find the journal name in the above links, provide the full name of the journal and highlight it in yellow.**DO NOT USE POINTS IN JOURNAL ABBREVIATIONS.**

Ota RR, Deprá GC, da Graça WJ, Pavanelli CS. Peixes da planície de inundação do alto rio Paraná e áreas adjacentes: Revised, annotated and updated. Neotrop Ichthyol. 2018; 16(2):e170094. http://dx.doi.org/10.1590/1982-0224-20170094

Note: You must provide only the e-location if there are no page numbers.

Sawakuchi AO, Hartmann GA, Sawakuchi HO, Pupim FN, Bertassoli DJ, Parra M, *et al.* The Volta Grande do Xingu: Reconstruction of past environments and forecasting of future scenarios of a unique Amazonian fluvial landscape. Sci Drill. 2015; 20:21–32. https://doi.org/10.5194/sd-20-21-2015

Note: You can use *et al.* for articles with more than six authors, and the page numbers separated by an **n-dash** (to automatically create n-dash in Word type "something – something" *(number-space-hyphen-space-number))*.

Abudayah WH, Mathis A. Predator recognition learning in rainbow darters *Etheostoma caeruleum*: specific learning and neophobia. J Fish Biol. 2016; 89(3):1612–23. https://doi.org/10.1111/jfb.13061

Note: You must present only **two** digits for last page if the previous digits coincide with the previous digits of the first page, separated by an **n-dash** (to automatically create n-dash in Word type "something – something" *(number-space-hyphen-space-number)*).

Koike Y, Koya Y. Viable periods of fertilizability of eggs and sperm of Japanese medaka, *Oryzias latipes*. Japan J Ichthyol. 2014; 61(1):9–14. Available from: https://www.jstage.jst.go.jp/article/jji/61/1/61_9/_pdf

Note: You must provide the issue number, and can present the URL of online articles without DOI number, preceded by Available from:..

Journal article – in press

Birindelli JL, Britski HA, Provenzano F. New species of *Leporinus* (Characiformes: Anostomidae) from the highlands of the Guiana Shield in Venezuela. Neotrop Ichthyol. Forthcoming 2019.

Note: You must cite only if the paper is about to be published.

Reports and other Government Publications

Eayrs S. A Guide to bycatch reduction in Tropical shrimp-trawl fisheries [Internet]. Rome; 2007. Available from: www.fao.org/docrep/015/a1008e/a1008e.pdf

International Commission on Zoological Nomenclature (ICZN). International code of zoological nomenclature. 4th ed. London: International trust for zoological nomenclature Natural History Museum [Internet]. London; 1999. Available from: https://www.iczn.org/the-code/the-international-code-of-zoological-nomenclature/

International Union for Conservation of Nature (IUCN). Standards and petitions subcommittee. Guidelines for using the IUCN Red List categories and criteria. Version 13 [Internet]. Gland; 2017. Available from: http://cmsdocs.s3.amazonaws.com/RedListGuidelines.pdf

Thesis

Oliveira AG. Predizendo impactos das mudanças climáticas sobre a diversidade funcional de peixes de água doce: um panorama "down under". [PhD Thesis]. Maringá: Universidade Estadual de Maringá; 2018. Available from: http://nou-rau.uem.br/nourau/document/?code=vtls000228862
Note: You must provide a hyperlink when available.

Figueiredo PICC. Decifrando a relação evolutiva entre *Gymnogeophagus labiatus* (Hensel, 1870) e *Gymnogeophagus lacustris* Reis & Malabarba 1988 (Cichlidae: Geophagini). [Master Dissertation]. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2018.

Webpages

Fricke R, Eschmeyer WN, Van der Laan R. Eschmeyer's catalog of fishes: genera, species, references [Internet]. San Francisco: California Academy of Science; 2019. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Taxonomic style instructions

This summary provides information specific to taxonomic manuscripts.
For large taxonomic revisions and reviews, see additional recommendations below. Items with * are required, others are recommended whenever applicable. Taxon accounts should be in alphabetical order. For original descriptions, the words "**new genus**" or "**new species**" should appear after the name of the new genus or species, preceded by a comma. The designation also must to appear in the caption of the holotype's figure, in the case of a new species. For species mentioned in the Diagnosis section but for which no comparative material was examined, please formally cite their original descriptions and provide the full references.

Note: Prior to submitting a description of a new taxon, please register new nomenclatural act(s) and the paper (as unpublished manuscript) at URL: http://zoobank.org/ and provide both the pub and the nomenclatural act codes just below the new taxon name. ***E.g.:***

urn:lsid:zoobank.org:**pub**:XX9XX9XX-X1X2-99XX-9X19-

9XXX0XX99X12

urn:lsid:zoobank.org:**act**:XX9XX9XX-X1X2-99XX-9X19-9XXX0XX99X12

Note: The pub number is only one for each manuscript, but, in case of more than one nomenclatural act, provide all the numbers.

Note: After publication, you must update the ZooBank article status from unpublished to published. This must be done by the author who made the initial registration.

Generic accounts

Order of presentation:

***Genus* Author, year (or new genus; do not abbreviate) (bolded and centered)**

urn:lsid:zoobank.org:act:XX9XX9XX-X1X2-99XX-9X19-9XXX0XX99X12
(in case of new genus only) (centered)

Synonymy.
Type species.*
Diagnosis.*
Description.*
Etymology. for new genus only*
Remarks.
Key to species.

Comments on the above:

Synonymy

Provide a complete synonymy listing all validly published names that have been applied to the genus, including all references in systematic treatments or identification guides that can help link the present concept of the genus to past concepts. The senior synonym is usually the correct and valid name. If applicable, include invalid names and mistakes in identity with suitable annotation to indicate their nature. For each name listed, include minimally: the original form of the name; the author and date of publication; reference and page number; basic information on the genus in the paper cited (modified from Wiley EO, Lieberman BS. Phylogenetics: theory and practice of Phylogenetic Systematics. 2nd edition. Hoboken: Wiley-Blackwell; 2011). Provide full references of all listed sources as part of the References section. If applicable, discuss the synonymy and cite relevant literature in the Remarks section. ***E.g.:***

Parodon Valenciennes, 1849

Parodon Valenciennes, in Cuvier, Valenciennes, 1849:50 (original description; type-species by original designation and monotypy: *Parodon suborbitalis* Valenciennes). —Günther, 1864:31(redescription). —Eigenmann, 1912:274 (diagnosis). —, Miles, 1943:251 (diagnosis in key). —Schultz, 1944:288 (diagnosis in key). —Campos, 1945:440 (diagnosis). —Miles, 1947:132 (diagnosis). – Travassos, 1955:4 (synonymic list). —Böhlke, 1958:83 (comments).—Ringuelet *et al*., 1967:180 (diagnosis in key). —Roberts, 1974b:433

(osteology). —Godoy, 1975:451 (diagnosis in key). —Géry, 1977:202 (diagnosis in key). —Britski *et al*., 1988:26 (diagnosis in key).

Nematoparodon Fowler, 1943:226 (original description; type-species by original designation and monotypy: *Parodon apolinari* Myers).
Note: Precede each quotation with an **m-dash** (Type an mdash using **Shift-Option-hyphen** on a Mac. In Windows, use **ALT + 0151***)*.

Type-species
For proposed new genera, the original name of the proposed typespecies, followed by author and year of publication (or new species) is sufficient. For previously proposed generic names, the following additional information is required (in this order): Nature of type designation (***e.g.***, original designation, monotypy, absolute tautonymy, etc). Whether the type-species was not designated in the original publication, the author, year and page of the subsequent designation should be cited (***e.g.***,Type by subsequent designation by Jordan, 1919: 45).

Diagnosis

Diagnosis should NOT be written in telegraphic style (for purposes of clarity). A generic diagnosis should preferably list the unique synapomorphies of the genus, followed by homoplastic derived characters and/or other useful distinguishing characteristics.

Description

In telegraphic style (*i.e.*, no verbs nor articles).

Etymology

For new names, state the gender, even though it may be obvious from the construction. Do not give an etymology for preexisting names. If it is necessary to discuss the etymology of an old name (for example, to justify an interpretation of its gender), include that in the Remarks section.

Key to species
If a key for identification of species is provided and it was not mentioned in the title, add "dichotomous key" or "identification key" as a keyword.

Specific accounts

Order of presentation:

Species **Author, Year (or new species - do not abbreviate) (bolded and centered)**

urn:lsid:zoobank.org:act:XX9XX9XX-X1X2-99XX-9X19-9XXX0XX99X12
(in case of new species only)
(centered)

Synonymy.
Holotype.* for new species only; include full collection data (see details, below)

Paratype(s). for new species only; include full collection data (see details, below)
Non-types. for new species only; include reduced collection data (see details, below) (Justification for separating non-types from types should be provided in the Material and Methods section)
Diagnosis.* see below for instruction on how to prepare a species diagnosis
Description.*
Coloration in alcohol.*
Coloration in life.
Sexual dimorphism.
Geographical distribution.*
Ecological notes.
Etymology. for new species only*
Conservation status. for new species only*
Remarks.
Material examined. for accounts of previously named species

Comments on the above:

Synonymy

Provide a complete synonymy listing all validly published names that have been applied to the species, including all references in systematic treatments or identification guides that can help link the present concept of the species to past concepts. The senior synonym is usually the correct and valid name. If applicable, include invalid names and mistakes in identity with suitable annotation to indicate their nature. For each name listed, include minimally: the original form of the name; the author and date of publication; reference and page number; country or basin and basic information on the species in the paper cited (modified from Wiley EO, Lieberman BS. Phylogenetics: theory and practice of Phylogenetic Systematics. 2nd edition. Hoboken: Wiley-Blackwell; 2011). Provide full references of all listed sources as part of the References section. If applicable, discuss the synonymy and cite relevant literature in the Remarks section. ***E.g.:***

Parodon caliensis Boulenger, 1895

Parodon caliensis Boulenger, 1895:480 (original description; typelocality: near Cali, Colombia). —Eigenmann, 1922(reprint 1976):109 (*partim*; Paila, río Cauca basin; diagnosis in key). —Miles, 1943:47 (río Cauca; redescription). —Miles, 1947:132 (río Magdalena; meristics). — Roberts, 1974b:416 (osteology; osteological illustrations). —Roberts, 1975:269 (dentition).

Parodon saliensis [sic]. —Roberts, 1975:269 (dentition).

Parodon Parodon caliensis. —Géry, 1977:203 (diagnosis in key).
Note: Precede each quotation with an **m-dash** (Type an mdash using **Shift-Option-hyphen** on a Mac. In Windows, use **ALT + 0151***)*.

Type-material

For new species, list types separately from other comparative material examined. Indicate when you have cleared and stained (c&s) specimens or genetic sequences in some online depository. Type specimens for which common barcoding sequences are available (***e.g.*** COI, Cytb, 12S, 16S) at an online depositary should be indicated clearly either in a table or in the text of the Holotype or Paratype(s) sections. In either case the following should be included:

The museum acronym and lot number, the maker gene/locus (***e.g.*** COI), the name of the depository (***e.g.*** GenBank), and the depositary accession number. Studies that include multiple sequences for phylogenetic or other analyses should list these along with the depositary name and accession number in a table or supplementary document (see GENETIC SEQUENCES in Author Instructions). It is acceptable for authors to indicate sequences in online depositories as "pending", but following acceptance of a manuscript, these numbers must be made available as a condition for final publication. ***E.g.:***

Group paratypes by country or basin, in alphanumeric order of museum acronym and catalog numbers inside each group. Include full collection data, in the following order:

Museum acronym and catalog number, number of specimens (except for holotype), size range separated by an **n-dash** (to automatically create n-dash in Word type "something – something" *(number-spacehyphen-space-number)*, number and size range of measured specimens, if different (in parentheses along with size range) locality (country, state, municipality, locality, basin, coordinates), date of collection [in dd, Month (3 letter abbreviation) and yyyy], and collector(s). ***E.g:***

Paratypes. LIRP 5640, 25, 38.5–90.3 mm SL (12, 75.0–90.3 mm SL), Brazil, São Paulo, Município de Marapoama, rio Tietê basin, ribeirão Cubatão at road between Marapoama and Elisiário, 21º11'35"S 49º07'22"W, 10 Feb 2003, A. L. A. Melo.

Note: Except in cases where no actively-curated scientific research collection exists, Holotypes must be deposited in collections in the country of origin of the species. When a species occurs in multiple countries, the holotype must be deposited in the country of the typelocality, with paratypes distributed among countries in which the species occurs. Even in cases of species endemic to one country, we encourage dissemination of paratypes.

Diagnosis

Do NOT write the diagnosis in telegraphic style (for purposes of clarity). A species diagnosis is typically a paragraph constructed of full sentences that list the most important traits that allow the reader to unequivocally identify the species. Ideally, the diagnosis includes one or more features that are unique to the species, preferably autapomorphic characters. If unique features were not discovered, the next best option is a differential diagnosis, within which a series of direct comparisons are made among species and the alternative character states specified by contrasts are stated explicitly (using "*vs.*" followed by the condition found in the species, or group of species, being compared, for each diagnostic feature). Diagnoses that consist only of a combination of characters (*i.e.*, traits listed sequentially which, when considered together, distinguish the species from congeners) should be avoided.
Note: In the event of listing species in the diagnosis without associated comparative material, please formally cite their original descriptions and provide full references.

Description

Write the description section in telegraphic style (*i.e.*, without verbs and articles). Treat bilaterally paired structures in the singular (***e.g.***, pelvic fin short, not pelvic fins short). Compound adjectives that include a noun should be connected by a hyphen (***e.g.***, pectoral-fin spine, NOT pectoral fin spine). Fin-ray formulae should be reported with unbranched rays in lower case Roman numerals, spines in upper

case Roman, and branched rays in Arabic numerals. Transitions between different types of rays should be indicated by a comma (,) and not a plus sign (+), or dash (-). We treat the catfish spinelet as a spine, so dorsal fin counts that include a spinelet should be reported as II,6 (or whatever the branched ray count is). ***E.g.:***

iii,7 or II,9. Not iii–7 or iii+7 (no spaces should be inserted after the comma).

Note: Do not include space between numerals and % (***e.g.,*** 25%, not 25 %).

Coloration

Write this section in telegraphic style (*i.e.*, without verbs or articles). This section may be divided into Coloration in alcohol and Coloration in life.

Geographic distribution

Geographic descriptors must NOT be translated and should be capitalized or not according to the standard usage in the language in question. English usage typically uses capitals (***e.g.***, Amazon River, Parana River, Paraguay River) while Portuguese and Spanish do not (***e.g.***, rio Solimões, río Magdalena, rio Paraná, río Parana, río Paraguay, rio Paraguai). When referring to a municipality or geopolitical region that includes the names of a water body, capitalize the entity as a proper noun in all languages (***e.g.***, Municipality of Arroio dos Ratos, State of Rio Grande do Sul).

Etymology

For new names, state the usage (adjective, noun, patronym, etc.), even though it may be obvious from the construction. For more information, see article 31 of the online International Code of Zoological Nomenclature (https://www.iczn.org/the-code/theinternational-code-of-zoological-nomenclature/the-code-online/). Do not provide an etymology for preexisting names, unless the etymology is necessary to justify the spelling. In such cases, include this information in the Remarks Section and not as a separate heading.

Conservation status

Please consider providing the conservation status, at least for new species, based on the IUCN criteria and categories. ***E.g.:***

Conservation status. Provide information on the conservation status assessment and finalize with… According to the International Union for Conservation of Nature (IUCN) categories and criteria (IUCN Standards and Petitions Subcommittee, 2017 [or later]), *Genus species* can be classified as Category (category abbreviation)].

Note: In such case, provide the full reference in the References.

Material examined

Provide only taxa, museum acronym, catalog number, number of specimens and size range. Indicate any types by: (Holo- Syn-, etc.) type of *Genus species* Author, date. For lectotypes or neotypes, also cite the source of designation. Specimen lots should be arranged taxonomically, and then by country or basin (in bold), in alphanumeric order of museum acronym and catalog numbers inside each group proposed. ***E.g.:***

Auchenipterichthys coracoideus: **Peru**: CAS 220574, 2, 104.0–107.0 mm SL, syntypes of *Trachycorystes coracoideus* Eigenmann, Allen, 1942).

Note: Deviation from this order is permissible only if an alternate arrangement shortens the text. If another arrangement is chosen, its use must be explained and justified in the Material and Methods section. List material of non-focal species as **Comparative material examined**, using the same rules of arrangement stated above.

Large taxonomic revisions and reviews

Before presenting the taxonomic accounts, provide a table at the beginning, cited early, that lists all the species included in the revision that are new and those that are being redescribed. Taxon accounts can be arranged in two ways: presenting the new species descriptions first (in alphabetic order) and then the redescribed species (in alphabetic order), OR reporting all the species in alphabetic order without separating new and redescribed ones. In either case, mentioning the words **new genus** or **new species** after the name of each new taxon presented, preceded by a comma. The chosen order of presentation should focus on brevity and comprehensibility.

Further information

Contact the Editor at neoichth@nupelia.uem.br.

ANEXO B – REPRESENTAÇÃO DA ESPÉCIE *Microphis lineatus* (POR JÚLIA D'OLIVEIRA, 2020)