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Morphological and molecular evidence for the occurrence of three *Hippocampus* species (Teleostei: Syngnathidae) in Brazil

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Abstract

For many decades only two species of seahorses were recognized from Brazil: *Hippocampus reidi* Ginsburg, 1933, the long snout seahorse, and *H. erectus* Perry, 1810, the lined seahorse. The presence of a possible third species, recognized in 2002, brought about the need for a broad revision of the genus in Brazilian waters. A total of 335 specimens of seahorses, obtained from Brazilian and other collections, representing the three putative species from Brazil were analyzed: *H. reidi*, the species of greatest abundance and occurs in estuaries and the sea; *H. erectus*, which occurs only in the sea, and *Hippocampus patagonicus* was also determined to be present based on multiple specimens. Our morphometric / numerical and molecular analysis showed that the species currently identified as *H. erectus* in Brazil is actually *H. patagonicus* Piacentino & Luzatto, 2004. The existence of a possible third species, was instead based on the true *H. erectus*, as confirmed in the present study by the study of classical systematic and mitochondrial analysis. Thus, we recognize three species of seahorses in Brazil: *H. erectus*, *H. reidi* and *H. patagonicus*.

Key words: Brazilian seahorses, morphometric analysis, COI gene, *Hippocampus erectus* complex

Resumo

Por muitas décadas, somente duas espécies de cavalos-marinhos foram reconhecidas no Brasil: *Hippocampus reidi* Ginsburg, 1933, o cavalo-marinho do focinho longo, e *H. erectus* Perry 1810, o cavalo-marinho raiado. A presença de uma possível terceira espécie, registrada em 2002, trouxe a necessidade de uma ampla revisão do gênero. Um total de 335 espécimes de cavalos-marinhos obtidos de instituições brasileiras e estrangeiras, representando as três possíveis espécies ocorrentes no Brasil foram analisadas: *H. reidi*, espécie de maior abundância com uma distribuição em estuários e oceanos; *H. erectus*, espécie com distribuição restrita ao mar. A presença de *H. patagonicus* foi avaliada pelo exame de vários animais. Nossas analises revelaram que a espécie identificada como *H. erectus*, no Brasil, é na verdade, *Hippocampus patagonicus* Piacentino & Luzzatto, 2004. As recentes aparições de uma terceira espécie representam a presença do verdadeiro *H. erectus*, como demonstrado no presente estudo, através das análises morfométricas e numéricas e análise mitocondrial. Assim, reconhecemos três espécies de cavalos-marinhos no Brasil: *H. erectus*, *H. reidi* e *H. patagonicus.*

Palavras-chave: cavalos-marinhos brasileiros, análise morfométrica, gene COI, complexo *Hippocampus erectus*

Introduction

The genus *Hippocampus* is composed of 55 species (Froese & Pauly, 2013) with a worldwide distribution. Several studies have pointed out that the taxonomy of the group is very problematic, with many species poorly defined

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Hippocampus patagonicus from Brazil and from Argentina do not only share similar morphological characteristics, but also genetic sequences as described by Luzzatto *et al*. (2012) in a study that compared sequences of cytochrome *b* (1144 bp) of *H. patagonicus* collected in San Antonio Bay (type locality) and at the Mar del Plata dock (Argentina) to a single specimen collected in Brazil (GenBank: AF192660). This Brazil sample was used by Casey *et al.* (2004) and named *H. erectus*; the two groups are different by only six base pairs, giving a genetic distance of 0.0053. This value is extremely low when compared to the interspecific distances found in our study (Table 3), confirming that the two groups belong to the same species, as suggested by Luzzatto *et al.* (2012), and probably represent interpopulational differences between two geographic populations.

The interspecific distance of *H. hippocampus* and *H. erectus* was similar between our data and those reported by Casey *et al.* (2004), namely 0.032 and 0.038, respectively. Due to the low interspecific genetic distance among *H. erectus, H. hippocampus* and *H. patagonicus*, the high bootstrap level (100%) that supported each monophyletic clade, confirmed that *H. patagonicus* belongs to the *H. erectus* complex and that they possibly have a more recent common ancestor (Casey *et al*., 2004*;* Boehm *et al.*, 2013). However, our data could not separate our samples of *H. reidi* from *Hippocampus algiricus* and *Hippocampus capensis* (Figure 4). The distance between *H. reidi* and *H. algiricus was* only d = 0.016 ± 0.003 and that between *H. reidi* and *H. capensis* was d = 0.018 ± 0.004. Casey *et al.* (2004) in a study employing cytochrome b found similar distance values between *H. reidi* and *H. algiricus* and a value two times higher between *H. reidi* and *H. capensis*. On the other hand, comparisons between other species pairs were about ten times higher (Table 3) in our study.

Most intraspecific pairwise distances reached a maximum of 0.018, in agreement with intraspecific values observed in other fish species (Waters & Burridge, 1999). In the BOLD database sequences are clustered using an algorithm called Barcode Index Number (BIN) that provide operational taxonomic units that closely correspond to species (Ratnasingham & Hebert, 2013). An analysis of BIN numbers in BOLD revels that all species have just one BIN number, with the exception of *H. reidi* which has two. Several studies have shown that most fish species can be separated from closely related species by a genetic distance of about d = 0.02, although there are several exceptions (Hubert *et al.*, 2008; Ward, 2009; Ward *et al.*, 2009; April *et al.*, 2011; Mabragaña *et al.*, 2011; Pereira *et al.*, 2013). Almost all species analyzed herein showed genetic distance values higher then d = 0.02; but for three species pairs (involving *H. reidi*, *H. capensis* and *H. algiricus*) the values were lower than this. Some possible explanations for this low genetic divergence are: (1) the recent speciation of some species groups (Ward *et al.*, 2009), (2) a possible variation in the COI mutation rate between different taxa (Krieger & Fuerst, 2002; Frézal & Leblois, 2008); and (3) possibly underlying problems.

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APPENDIX 1. Material examined.

Hippocampus erectus: 85 specimens, 59–120 mm Ht. USA, Florida: MCZ 11684; MCZ 51551; RMMU 2337c; RMMU 2337d RMMU 2337a; RMMU 2337b; RMMU 2337e; USNM 233097; USNM 383982; TCWC 7293; TCWC 7294(2); TCWC 7294(2); TCWC 7295, TCWC 7311; TCWC 7312; TCWC 7315; TCWC 7331(2); TCWC 7338; TCWC 7978; TCWC 10649; USNM 84598; USNM 86117; USNM 89786; USNM 107378; USNM 112511; USNM 124849; USNM 155441; USNM 161345 (2); MCZ 51529. CARIBBEAN: TCWC 6675. CUBA: MCZ 11663; MCZ 11664. PORTO RICO: MCZ 51547. BRAZIL, Pará: MZUSP 3213 (2). Rio Grande do Norte: LAPIBE/UFRN S/N. Pernambuco: MCZ 158653; MZUSP 10650; PH 059e; PH 036e; PH 045e; PH 064e; PH 033e; PH 058e; HP 069e; PH 028e; PH 078e; PH 122e; PH 079e; PH 080e; PH 081e; PH 256e; PH 001e; PH 082e; PH 083e; PH 084e; PH 085e; PH 060e; PH 071e; PH 035e; PH 041e; PH 047e; PH 043e; PH 042e; PH 072e; PH 054e; PH 046e; PH 040e; PH 077e; PH 037e; PH 013e; PH 030e; PH 011e; PH 034e; PH 067e, PH 017e; PH 086e; PH 020e; PH 065e; PH 015e; PH 007e; PH 002e; PH 026e; PH 029e; PH 018e; PH 044e; PH 005e; PH 008e; PH 006e; PH 021e; PH 019e; PH 049e; PH 009e; PH 012e; PH 031e; PH 014e; PH 038e; PH 010e; PH 023e; PH 022e; PH 003e; PH 025e; PH 051e; PH 057e. BAHIA: UFBA 1401; MNRJ 22159; Rio de Janeiro: MCZ 11667; MCZ 11668; PH 424e. São Paulo: MZUSP 2791; MZUSP 3215; MZUSP 87221; MZUSP 87228. Santa Catarina: MCZ 11674 (2). Rio Grande do Sul: PH 466e. ARGENTINA: MCZ 11669.

Hippocampus reidi: 104 specimens, 68 a 188 mm Ht. BRAZIL, Amazonas: MCZ 168387; MCZ 59348; MCZ 59349; MCZ 59350; MZUSP 1174; MZUSP 847 (2). Pará: MPEG 11244; MPEG 3846. Ceará: PH 718r. Rio Grande do Norte: LAPIBE/ UFRN S/N (3); UNESP 26013; UNESP 26014; UNESP 26015; UNESP 26016; UNESP 26017; PH 760r; PH 761r; PH 762r; PH 763r; PH 764r; PH 765r; PH 766r; PH 767r; PH 768r; PH 769r; PH, 770r; PH 771r; PH 772r; PH 773r. Pernambuco: MCZ 158435; MZUSP 10651; PH 018r; PH 019r; PH 020r; PH 113r; PH 114r; PH 115r; PH 116r; PH 117r; PH 123r; PH 124; PH 125r; PH 126r; PH 127r; PH 128r; PH 129r; PH 130r; PH 131r; PH 132r; PH 133r; PH 134r; PH 135r; PH 136r; PH 145r; PH 172r; PH 173r; PH 174r; PH 175r; PH 176r; PH 177r; PH 178r; PH 179r; PH 180r; PH 181r; PH 182r. Alagoas: PH 021r; PH 022r; PH 023r; PH 024r; PH 025r; PH 026r; PH 027r; PH 028; PH 029; PH 050r; PH 051r; PH 052r; PH 053r; PH 054r; PH 055r; PH 056r; PH 057r; PH 058r; PH 018r; PH 019r; PH 020r; H 059r; PH 060r; PH 061r; PH 062r; PH 063r; PH 064r; PH 065r; PH 066r; PH 068r; PH 069r; PH 070r; PH 071r; PH 072r; PH 073r; PH 074r; PH 075r; PH 076r; PH 078r; PH 079r; PH 080r; PH 081r; PH 082r; PH 083r; PH 084r; PH 085r; PH 086r; PH 087r; PH 088r; PH 089r; PH 090r; PH 092r; PH 094r; PH 095r; PH 096r; PH 097r; PH 098r; PH 099r; PH 100r; PH 101r; PH 102r; PH 103r; PH 104r; PH 105r; PH 106r; PH 107r; PH 108r; PH 109r; PH 110r; PH 111r; PH 112r. Bahia: UFBA 1401; MCPUCRS 4870 (2); MCZ 11686. Espirito Santo: MNRJ 6844. Rio de Janeiro: MCZ 4721; MCZ 59348; MNRJ 1547; MCZ 11665; MZUSP 850; MZUSP 851 (2). São Paulo: MCPUCRS 7661; MCPUCRS 7662; MZUSP 784; MZUSP 852. Paraná: PH 50r; PH 51r. Santa Catarina: MCPUCRS 1181; MCPUCRS 2869; MCPUCRS 7328; MCPUCRS 7687; PH 105r. Rio Grande do Sul: MCPUCRS 4348.