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

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.
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 reveals 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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References

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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 7397; TCWC 7464; USNM 84598; USNM 86117; USNM 89786; USNM 107311; 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 060e; PH 061e; PH 062e; PH 063e; PH 064e; PH 065e; PH 066e; PH 067e; PH 068e; PH 069e; PH 070e; PH 071e; PH 072e; PH 073e; PH 074e; PH 075e; PH 076e; PH 077e; PH 078e; PH 079e; PH 080e; PH 081e; PH 082e; PH 083e; PH 084e; PH 085e; PH 086e; PH 087e; PH 088e; PH 089e; PH 090e; PH 091e; PH 092e; PH 093e; PH 094e; PH 095e; PH 096e; PH 097e; PH 098e; PH 099e; PH 100e; PH 101e; PH 102e; PH 103e; PH 104e; PH 105e; PH 106e; PH 107e; PH 108e; PH 109e; PH 110e; PH 111e; PH 112e. Bahia: UFBA 1401; MCPUCRS 4870 (2); Pará: MPEG 11244; MPEG 11245. Ceará: PH 718r. Rio Grande do Norte: LAPIBE/UFRRN S/N. Pernambuco: MCZ 158435; MZUSP 10651; PH 018r; PH 019r; PH 020r; PH 021r; PH 022r; PH 023r; PH 024r; PH 025r; PH 026r; PH 027r; PH 028r; PH 029r; PH 030r; PH 031r; PH 032r; PH 033r; PH 034r; PH 035r; PH 036r; PH 037r; PH 038r; PH 039r; PH 040r; PH 041r; PH 042r; PH 043r; PH 044r; PH 045r; PH 046r; PH 047r; PH 048r; PH 049r; PH 050r; PH 051r; PH 052r; PH 053r; PH 054r; PH 055r; PH 056r; PH 057r; PH 058r; PH 059r; PH 060r; PH 061r; PH 062r; PH 063r; PH 064r; PH 065r; PH 066r; PH 067r; PH 068r; PH 069r; PH 070r; PH 071r; PH 072r; PH 073r; PH 074r; PH 075r; PH 076r; PH 077r; 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 091r; PH 092r; PH 093r; 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 11668. Espirito Santo: MNRJ 6844. Rio de Janeiro: MCC 4721; MCC 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.

**Hippocampus reidi**: 104 specimens, 68–188 mm Ht. BRAZIL, Amazonas: MCZ 168387; MCZ 59348; MCZ 59349; MCZ 59350; MCPUCRS 1174; MCPUCRS 847 (2). Pará: MPEG 11244; MPEG 3846. Ceará: PH 718r. Rio Grande do Norte: LAPIBE/UFRRN S/N. Pernambuco: MCZ 158435; MZUSP 10651; PH 018r; PH 019r; PH 020r; PH 021r; PH 022r; PH 023r; PH 024r; PH 025r; PH 026r; PH 027r; PH 028r; PH 029r; PH 030r; PH 031r; PH 032r; PH 033r; PH 034r; PH 035r; PH 036r; PH 037r; PH 038r; PH 039r; PH 040r; PH 041r; PH 042r; PH 043r; PH 044r; PH 045r; PH 046r; PH 047r; PH 048r; PH 049r; PH 050r; PH 051r; PH 052r; PH 053r; PH 054r; PH 055r; PH 056r; PH 057r; PH 058r; PH 059r; PH 060r; PH 061r; PH 062r; PH 063r; PH 064r; PH 065r; PH 066r; PH 067r; PH 068r; PH 069r; PH 070r; PH 071r; PH 072r; PH 073r; PH 074r; PH 075r; PH 076r; PH 077r; 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 091r; PH 092r; PH 093r; 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 11668. Espirito Santo: MNRJ 6844. Rio de Janeiro: MCC 4721; MCC 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.