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Scientific Note

Novel sex-related characteristics of the longsnout seahorse *Hippocampus reidi* Ginsburg, 1933

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This paper presents novel sex-related characteristics in *Hippocampus reidi*, expressed as a prominent and pigmented keel (males only), and by a row of dorsolateral spots mostly (97.4%) found in males. While the keel was found even in the smallest males sampled, the dorsolateral spots appear to be associated with body size. *Ex situ* observations revealed that during courtship males highlighted both characters by turning pale their background body colour. Understanding such colour and behavioural patterns is relevant to increase our knowledge about sexual selection in a morphologically and biologically unique group of fishes.

O presente trabalho descreve novos caracteres sexuais em *Hippocampus reidi*, expressos como uma quilha proeminente e pigmentada (machos apenas), e uma série de máculas dorsolaterais, encontradas principalmente (97,4%) em machos. Enquanto a ocorrência da quilha foi observada mesmo nos menores machos, a presença de máculas dorsolaterais parece estar associada ao tamanho do corpo. Observações *ex situ* mostraram que os machos realçam ambos os caracteres durante o comportamento de corte, ao tornar pálida a cor de base do corpo. Destaca-se a relevância da compreensão de tais padrões de colorido para o avanço no conhecimento relativo à seleção sexual em um grupo de peixes de morfologia e biologia singulares.

Key words: Syngnathidae, Colouration, Keel, Dorsolateral spots, Courtship.

Knowledge about sexual dimorphism is central for understanding the ecology, behaviour, and life history of a species, as well as for making morphological comparisons between populations (*e.g.*, Kitano *et al*., 2007). Many sexual dimorphisms are secondary changes in males, which generally are larger, often more colourful and more conspicuous, and more vocal than females (Darwin, 1871). Such secondary sexual characteristics are often interpreted as promoting success in male-male competition or female choice, and hence increasing opportunities for mating (Iguchi *et al*., 1991).

Seahorses (genus *Hippocampus*, family Syngnathidae) have a social structure that involves close pair-bonding, and are distinguished by extreme morphological specialization for paternal care, represented by a specialized pregnancy structure, the brood pouch. Aside from the pouch, however, these fishes reportedly tend to show no or little sexual dimorphisms (Foster & Vincent, 2004) other than those expressed as differences in body proportions. Sex-related disparity in colour patterns have seldom been described for seahorses, although dichromatism appears to be present in some species (see Lourie *et al*., 2004).

This paper describes novel sex-related characteristics of the longsnout seahorse *Hippocampus reidi* Ginsburg, 1933, based on specimens from Brazil. *Hippocampus reidi* is a slender-body species with variable colour patterns, consisting of at least six distinct background colours (Rosa *et al*., 2002), often profusely spotted with dark dots and numerous tiny white dots (especially on the tail), and may also have pale saddles across dorsolateral surfaces (see Lourie *et al*., 1999, 2004). As most seahorse species studied to date (see Foster & Vincent, 2004, for a review), *H. reidi* was inferred to be monogamous and forming pair bonds (Rosa *et al*., 2007). Published data on sex-related differences in the species are limited to the brood pouch and height (Foster & Vincent, 2004; Rosa *et al*., 2007).

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Data used in the present study were gathered from October 2005 to July 2007 at four estuaries in Northeastern Brazil: (1) the Camurupim/Cardoso estuarine complex (02°53'S-02°58'S, 41°24'W-41°28'W), Delta do Parnaíba Environmental Protection Area, State of Piauí, as described in Rosa *et al.* (2007) and Mai & Rosa (2009); (2) the Mamanguape estuary, Barra de Mamanguape Environmental Protected Area (06°43'S-06°51'S, 35°07'W-34°54'W), State of Paraíba (as described in Rosa *et al.*, 2007); (3) the Itapessoca estuary (07°37'S-07°41'S, 34°50'W-34°55'W , described in Rosa *et al.*, 2007) and (4) the rio Formoso estuary (08°35'S- 08°45'S, 35°5'W-35°10'W), both in the State of Pernambuco.

Daytime underwater censuses (50 X 1-2 m transects) were performed in depths ranging from 0.01-2.0 m. For each sighted specimen of *H. reidi*, data on colour patterns, height (following Lourie *et al.*, 1999), sex and reproductive state (following Foster & Vincent, 2004; Rosa *et al.*, 2007), and life stage (following Castro *et al.*, 2008) were recorded. To avoid the risk of classifying young males (which have no brood pouches) as females, all juveniles were placed in the category "undetermined sex". Specimens were carefully hand-picked, kept underwater throughout data collection and then returned to the same place where originally found. Seahorses were identified by a collar placed on the base of the tail (Felício *et al.*, 2006) to ensure that throughout the study all individual data came from different specimens. At the end of the study, an extensive effort was made to remove the marks.

Additional information was obtained from 21 pairs of captive reared specimens of *H. reidi* kept at the Laboratório de Peixes - Ecologia e Conservação (LAPEC), Universidade Federal da Paraíba, Brazil, between August 2007 and October 2008. Seahorses were observed using focal-animal sampling, following Lehner (1996). Each specimen was observed for a total of 60 min, during which all behaviours were recorded and filmed.

In order to assess diferences in the occurrence of the colour traits between sexes, life stages and reproductive states, χ^2 tests were performed. Juveniles were excluded from these analysis. Additionaly, comparisons between males height and the occurrence of colour traits were made using *t* test.

Examination of 1,406 specimens of *H. reidi* (582 males; 599 females; 225 juveniles) in the field revealed the existence of a novel sexual dimorphism in the longsnout seahorse (Fig. 1), expressed as an exclusive pattern consisting of a prominent and pigmented keel in males, and by a row of conspicuous dorsolateral spots on the trunk, mostly found in males. Marks appear as circled dark spots arranged dorsolaterally between adjacent trunk rings, on both sides of the body, and varied in number and colour intensity. All male seahorses examined in this study had the characteristic keel, from the smallest (8.8 cm height) to the largest specimen (19.3 cm), consisting of a sharp and dark edge along the ventral portion of the trunk.

Dorsolateral spots were found in 342 specimens, the vast majority of which (97.4%) were males ($\chi^2 = 452.32$, d.f. =1, $p < 0.001$; Fig. 2). Despite strongly related to male specimens, dorsolateral spots were found in seven females ($n_{total} = 599$) and two juveniles ($n_{total} = 225$). The occurrence of dorsolateral spots in males was significantly higher in adult specimens ($\chi^2 = 55.32$, d.f. = 1, $p < 0.001$), regardless of reproductive stage ($\chi^2 = 2.66$, d.f. = 1, $p = 0.10$). A positive correlation was also found between the occurrence of dorsolateral spots and mean height of adult males (Fig. 3), as follows: specimens with markings were significantly larger than those which did not exhibit that pattern ($t = -11.12$, d.f. = 580, $p < 0.001$).

Ex situ observations of 21 pairs of *Hippocampus reidi* revealed that during courtship the background colour of both males and females became pale (Fig. 4). In the males, this change highlighted the dark colouration of the keel and accentuated the dorsolateral spots against the paler background colour.

In general, morphology of seahorses is conservative and sexual dimorphism is mainly demonstrated by the brood pouch in males, besides body proportions and height (see Foster & Vincent, 2004). Other sex-related differences have been suggested to occur in a few species (see Table 1). Sexual dichromatism regarding dorsolateral spots, however, was only recorded for *H. trimaculatus* (Lourie *et al.*, 1999, 2004), *H. hippocampus* ("form *microstephanus*"), *H. manadensis* and *H. takakurai* (Kuiter, 2009), in which this character was found exclusively or more frequently in males (Table 1), as found in this study for *H. reidi*.

Differently from the dorsolateral spots, the prominent and pigmented keel was only found in males, corroborating the results of Barros (2005), who observed that females of *H. reidi* lacked a keel. Records of sexual dimorphism related to the latter characteristic - described as a sharp median ridge running down the ventral side of the trunk (Lourie *et al.*, 2004) - are uncommon in the genus *Hippocampus* (Table 1). Males with prominent keels have been recorded in *H. capensis*, *H. ingens*, *H. jayakari*, *H. histrix* (Lourie *et al.*, 1999, 2004), and *H. biocellatus* (Kuiter, 2009). In *H. abdominalis*, however, females' keel was usually deeper than in males (see Lourie *et al.*, 2004).

Our observation that *H. reidi* males highlight the dorsolateral marks and keel during courtship by turning their background colour into a paler pattern was anecdotally reported by Kuiter (2009) for a morphotype of *H. hippocampus*. Keel highlighting due to body colour brightening was also reported for males and females of *H. fuscus* during courtship (Vincent, 1990). Given that brightening of colours is a characteristic behaviour displayed during seahorse daily greetings and courtship (see Foster & Vincent, 2004), and that monogamy appear to be reinforced by these daily greetings (*H. fuscus*, Vincent, 1995; *H. whitei*, Vincent & Sadler, 1995; *H. zosterae*, Masonjones & Lewis, 1996), the display of the highlighted marks and keel observed in this study may be an important signal to communicate readiness to mate, although the occurrence of dorsolateral spots *per se* was not related to reproductive activity. Amplification of the normal contrast of colour patterns has also been recorded for females of the pipefish *Nerophis lumbriciformis* (Monteiro *et al.*, 2002), while in *Syngnathus typhle* and *Syngnathus abaster* both sexes amplified the normal contrast, in the

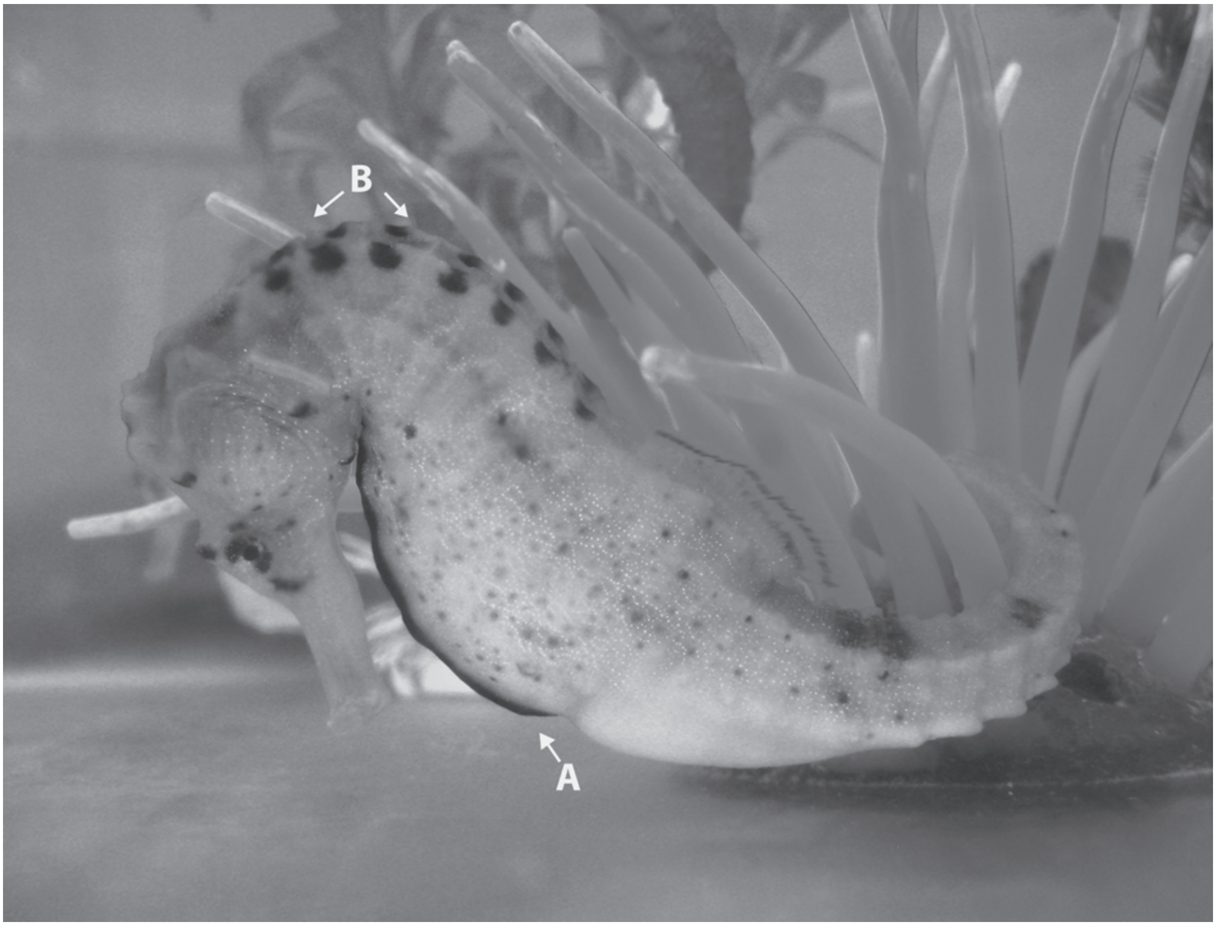

Fig. 1. A captive-reared male of *Hippocampus reidi*, showing (A) the prominent and pigmented keel and (B) dorsolateral spots. Photo credits: T. P. R. Oliveira.

context of courtship and competition (Berglund *et al*., 1997, 2005; Bernet *et al*., 1998; Silva *et al*., 2010). Given that both males and females seahorses pale their background colour during courtship, males should have distinct attractive patterns to call the attention of females – commonly the choosy sex in this group (see Foster & Vincent, 2004), although some species appear to show some plasticity in sex roles (*H. abdominalis*, Wilson & Martin-Smith, 2007; Mattle & Wilson, 2009; *H. guttulatus*, Naud *et al*., 2009). Mate choices often depend upon coloration and strongly affect both population divergence and speciation (Andersson, 1994). Therefore, it is not surprising that the use of colour patterns as sexual signals has been demonstrated in many other fish species (see Kodric-Brown, 1998).

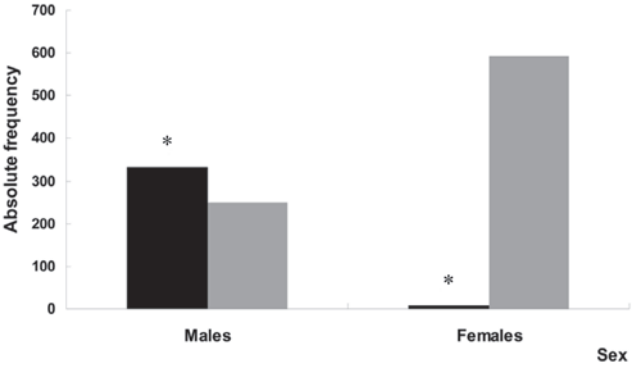

Fig. 2. Occurrence of dorsolateral spots according to sex in *Hippocampus reidi*. Specimens presenting dorsolateral spots (black bars); specimens without dorsolateral spots (grey bars). (*) Significant difference ($p < 0.001$).

The permanent striped pattern present in the pipefish *Syngnathus typhle* (present in both sexes) is increased in females during nuptial dance and intrasexual competition (Berglund *et al*., 1997; Bernet *et al*., 1998). Different studies have shown that this behaviour has important functions, such as predicting female mating success (Bernet *et al*., 1998), determining female dominance (Berglund & Rosenqvist, 2001), intimidating rival females (Berglund & Rosenqvist, 2009) enhancing reproductive success (Berglund *et al*., 1997), and serving as an amplifier of female body size (Berglund, 2000). With regards to *S. typhle* males, Berglund *et al*. (2005) found that specimens which displayed more benefited from this by receiving more eggs. Whether the colour patterns described here are used during male-male competition or for signaling male quality to females, it is unknown. Nevertheless, it is likely from our results that the patterns displayed by *H. reidi* represent a trade-off between decreased crypsis and increased conspicuousness. Adult seahorses are presumed to have few predators (Foster & Vincent, 2004), and therefore the highlighted colour patterns described in this study possibly do not enhance predation risk during reproduction. Describing a novel dimorphism in *H. reidi*, we hope to stimulate further research on biology, ecology and behaviour of seahorses.

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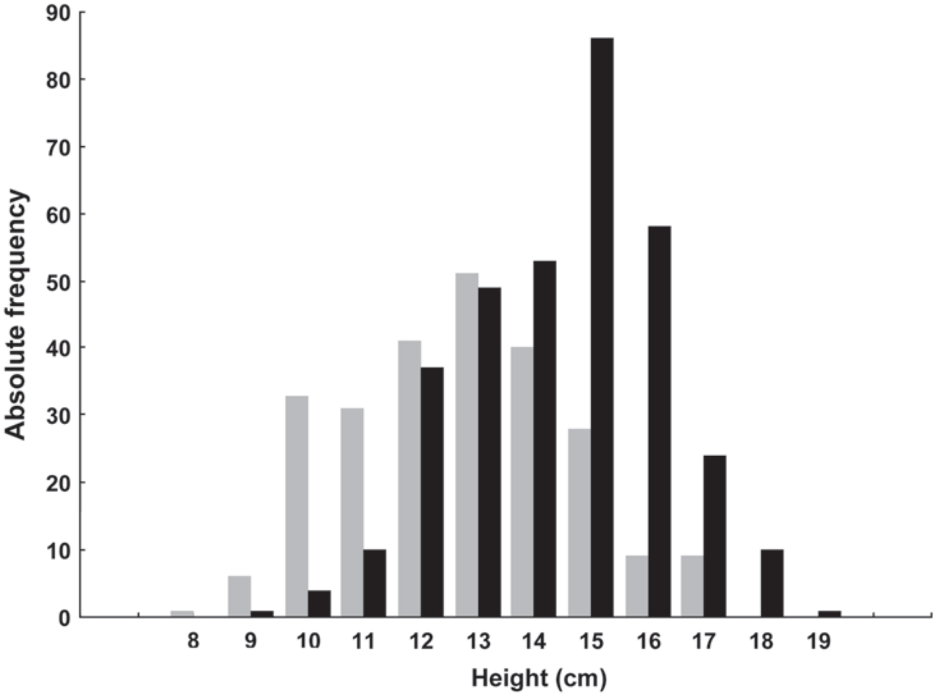

Fig. 3. Occurrence of dorsolateral spots according to height in males of *Hippocampus reidi*. Males presenting dorsolateral spots (black bars); males without dorsolateral spots (grey bars).

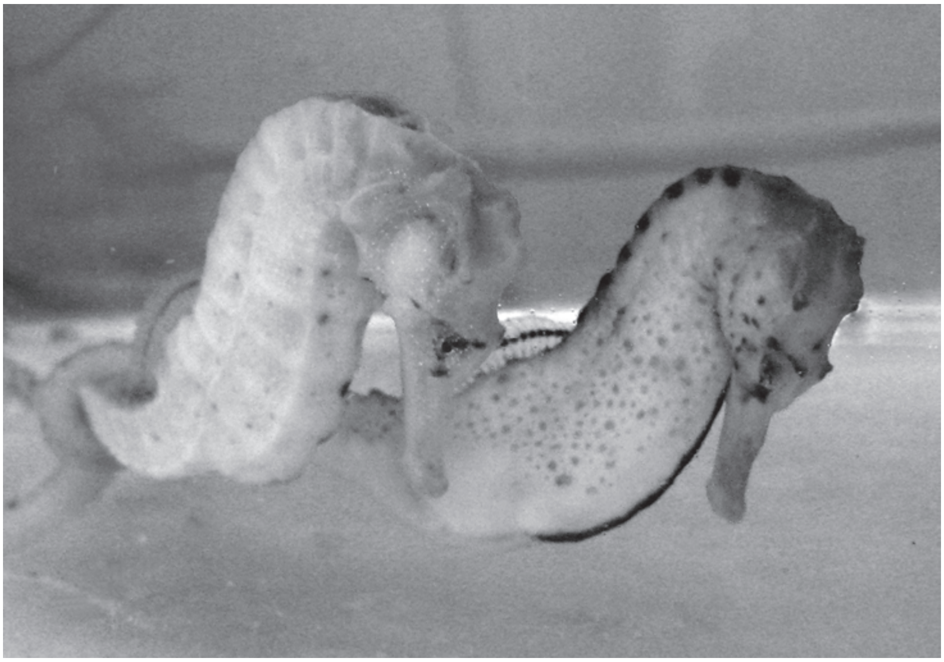

Fig. 4. A captive-reared pair of *Hippocampus reidi* with the male (right) showing highlighted dorsolateral spots and keel, during courtship. Photo credits: T. P. R. Oliveira.

Table 1. Occurrence of distinct colour patterns and sexual dimorphism in genus *Hippocampus*. 1 = Lourie *et al*. (1999); 2 = Lourie *et al*. (2004); 3 = Vincent (1990); 4 = Kuiter (2009); 5 = Jones & Avise (2001). NI = Not informed; M = male; F = female.

Species / Sex	Spotted patterns	Keel	Other traits	Sources
H. abdominalis / M and F	M with more dark blotches, and with a yellow slash near the top of the pouch	usually deeper in F than in M		1,2,3
H. biocellatus (*H. trimaculatus* complex) / M	Eye marks on the back in both sexes facing laterally	M with deep keel of soft skin along median ridge of the trunk		4
H. camelopardalis / NI	Dark spot on the top of head, and on 1st, 4th and 7th trunk rings (not always visible)			1,2
H. capensis / M		M commonly with a slight keel		1,2
H. dahli (*H. trimaculatus* complex) / M and F			Distinct colour patterns in M and F	4
H. hippocampus ("form *microstephanus*") / M	M with a series of black spots along upper ridges of trunk			4
H. histrix / M		Sharp, dark-edged keel especially on M		2
H. ingens / M and F	Sexually mature F often with dark patch bellow the anal fin	M commonly with prominent keel		1,2
H. jayakari / M		M with deeper keel		1,2
H. manadensis (*H. trimaculatus* complex) / M and F	M often with several distinctive dark spots along upper trunk ridges, outlined by a pale ventral edge		F occasionally show zebra pattern	4
H. sindonis / M and F			M with smooth coronet; F with fleshy extensions from spines above eye and coronet	4
H. spinosissimus / M			M blunt-tipped spines bordering pouch	1,2
H. subelongatus / NI			M and F differ in the frequency of colour morphs	5
H. takakurai (*H. trimaculatus* complex) / M	M with dark spots along upper trunk ridges			4
H. trimaculatus / M	M may have large dark spots on 1st, 4th and 7th trunk rings; spotted pattern more common in M			1,2

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