Genetic evidence and new morphometric data as essential tools to identify the Patagonian seahorse *Hippocampus patagonicus* (Pisces, Syngnathidae)

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A genetic study to support morphometric analyses was used to improve the description and validate the Patagonian seahorse *Hippocampus patagonicus* (Syngnathidae) on the basis of a large number of specimens collected in the type locality (San Antonio Bay, Patagonia, Argentina). DNA sequence data (from the cytochrome *b* region of the mitochondrial genome) were used to differentiate this species from its relatives cited for the west Atlantic Ocean. Both phylogenetic and genetic distance analyses supported the hypothesis that *H. patagonicus* is a species clearly differentiated from others, in agreement with morphometric studies. *Hippocampus patagonicus* can be distinguished from *Hippocampus erectus* by the combination of the following morphometric characteristics: (1) in both sexes and all sizes of *H. patagonicus*, the snout length is always less than the postorbital length, whereas the snout length of *H. erectus* is not shorter than the postorbital length in the largest specimens; (2) in both sexes of *H. patagonicus*, the trunk length:total length (L_{Tr}:L_T) is lower than in *H. erectus* (in female *H. patagonicus*: 0·27–0·39, *H. erectus*: 0·36–0·40 and in male *H. patagonicus*: 0·24–0·34, *H. erectus*: 0·33–0·43) and (3) in both sexes, tail length:total length (L_{Ta}:L_T) in *H. patagonicus* is larger than in *H. erectus* (0·61–0·78 *v*. 0·54–0·64).

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Key words: Argentina; phylogeny; redescription; San Matías Gulf; taxonomy.

INTRODUCTION

The genus *Hippocampus* Rafinesque 1810 (Syngnathidae) includes all recognized species of seahorses (Wilson & Vincent, 1999). Despite recent revisions (Lourie *et al.*, 1999*a*; Kuiter, 2003), there remains considerable taxonomic confusion within this group. This makes it difficult to implement seahorse management and conservation actions (Lourie *et al.*, 1999*a*) as well as related research. The peculiar morphology of

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seahorses and the absence of many typical features traditionally used by taxonomists have contributed to a lack of understanding of the taxonomic status of these fishes (Kuiter, 2001). Therefore, it is necessary to re-examine the material available and to revise the existing classification on a global basis.

Until 2003, only a single seahorse species had been reported from Argentinean waters (south-west Atlantic Ocean): *Hippocampus punctulatus* Guichenot 1853, a synonym of *Hippocampus erectus* Perry 1810 (Menni *et al.*, 1984). Recent revisions of the genus, however, have suggested that several species may exist in the neighbouring waters of Brazil (Lourie *et al.*, 1999*a*; Kuiter, 2003). In certain areas of San Matías Gulf (41° S–42° S; 63° W–65° W, Patagonia, Argentina), seahorse aggregations are regularly recorded, especially during spring and summer months. Specimens are commonly found in the clear, shallow waters of San Antonio Bay. Here, seahorses are sufficiently abundant to sustain a small-scale fishery that developed over the past three decades.

Until recently, *H*. *punctulatus* was misidentified as *H*. *erectus*. Preliminary studies carried out from 2001 to 2003 on specimens collected from San Antonio Bay (R. González, unpubl. data), however, showed that certain morphometric characteristics were different from those described for *H*. *erectus* by Vari (1982). Moreover, based on a preliminary examination, sampling data and colour images, Kuiter (2003) designated this seahorse as an undetermined species.

After these preliminary studies, a new nominal species, the Patagonian seahorse *Hippocampus patagonicus*, was described by Piacentino & Luzzatto (2004) with the type locality in San Antonio Bay. An exhaustive analysis of the diagnostic characteristics (both morphometric and meristic ones) proposed in their original description, however, does not allow a clear and easy differentiation of this new species, due to the high overlap between the ranges of body measurements for *H*. *patagonicus* and those described for *H*. *erectus* (Vari, 1982; Lourie *et al.*, 1999*b*). Recent studies, focused on the phylogeny of the *H*. *erectus* complex (Luzzatto *et al.*, 2012) and the biogeography of Atlantic seahorses (Boehm *et al.*, 2013), have assumed *H*. *patagonicus* as a valid species. In one of these papers, however, Luzzatto *et al.* (2012) pointed out that this status needed to be confirmed because the differentiation from *H*. *erectus* is not clear based on morphometric and meristic data published to date.

Although traditional studies based on meristic and morphometric characteristics are broadly used in fish taxonomy, it is well known that such approaches appear to be insufficient to perform unambiguous descriptions in seahorses. The singular morphology of this genus, the lack of key features (*e.g.* pelvic and caudal fins) usually used in the morphometric analysis of fishes and the particular variations in body proportions, which generally overlap among seahorse species (Lourie *et al.*, 1999*b*), make the process of defining a new species very difficult. In this sense, studies combining morphometric and genetic evidence offer the most robust approach in determining the taxonomy and phylogeny of seahorses (Lourie *et al.*, 1999*b*).

The main objective of the study presented here is to provide genetic evidence and unpublished morphometric data to expand and improve the original description and validate the taxonomic status of *H*. *patagonicus*. A new description of this species is presented on the basis of an exhaustive examination of morphometric characteristics and its relationships to other species of *Hippocampus*, providing a critical analysis of the original description. The conclusions are supported with genetic evidence. A robust and broad morphometric database was used, along with a phylogenetic
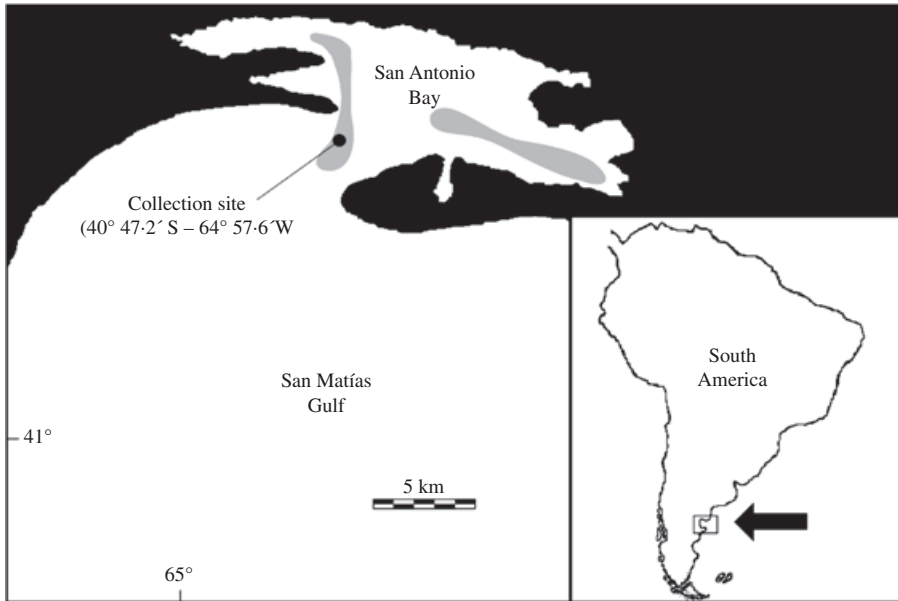
FIG. 1. Location of San Antonio Bay and San Matías Gulf. Collection site of the specimens (●) and main areas of distribution (▣) are indicated.

analysis to unambiguously differentiate and validate *H. patagonicus* from other seahorses from the west Atlantic Ocean biome.

MATERIALS AND METHODS

STUDY AREA

San Antonio Bay (Fig. 1) is an estuarine environment with semi-diurnal tides (tidal amplitude ranges between 6 and 9 m) and a system of channels exposed to strong tidal currents (Schnack *et al.*, 1996). Water temperature ranges from 6 to 8° C in winter to 25 to 28° C in summer. Water circulation is governed by tidal currents near the bay (Schnack *et al.*, 1996) and by the presence of one cyclonic gyre in the northern half of the gulf (Piola & Scasso, 1988). Both phenomena contribute to the retention of water masses in the northern area of the gulf and to differentiate this marine environment from the southern half of the gulf and from the neighbouring open sea. The area described above constitutes the main habitat of the *H. patagonicus*, being very scarce or rare outside the bay. Additionally, no other seahorse species has been recorded in the San Matías Gulf.

SAMPLING

Taxonomic analysis was performed using specimens collected during scuba surveys (2001–2009), in the context of a broad study involving biological features (*i.e.* growth, feeding habits, reproduction and parasitology) and population features of seahorses in San Antonio bay. Because several species of the genus *Hippocampus* are considered to be threatened according to the IUCN Red List of Threatened Species (IUCN, 2013) and the collection localities of *H. patagonicus* were within the Marine Protected Area of San Antonio Bay, it was essential to obtain permission to collect the species from the environmental authority of the Rio Negro Province (Consejo de Ecología y Medio Ambiente–CODEMA).

Specimens were collected along transects (50–70 m long × 1 m wide) and caught by hand at a depth between 2 and 6 m during low tides. All seahorses present in transects were captured and individually stored in plastic bags. Fishes were immediately killed by an overdose of tricaine methanesulphonate (MS-222) at 500 mg l^{-1} of sea water and brought to the laboratory to be measured and dissected.

MORPHOMETRIC AND MERISTIC STUDY

Specimens of *H. patagonicus* collected in surveys were dissected and examined in the laboratory using a stereomicroscope (×5 to ×25). The meristic characteristics examined were number of rays in the anal, pectoral and dorsal fins, and the number of rings on the trunk and tail. In order to compare them with *H. erectus*, measurements (0·01 mm) were recorded using a digital calliper following Vari (1982) (Fig. 2): total length (L_T, distance from the median groove of the coronet to the tip of the tail, with the tail straightened for measurement), trunk length (L_{Tr}, measured as total length to sub-dorsal spine), tail length (L_{Ta}, distance from the sub-dorsal spine to the tip of the tail), head length (L_H, distance from the tip of the snout to the gill opening), postorbital length (L_{PO}, distance from the ocular orbit to the gill opening), snout length (L_{Sn}, distance from the tip of the snout to the front of the ocular orbit) and orbital diameter (D_O, maximum distance between eye borders, measured on the line connecting the snout tip with the gill opening). Other morphological characteristics proposed by Lourie *et al.* (1999*a*) and utilized in the description are coronet index (I_C), chin shape index (I_{CS}) and spininess index (I_S). The mass of the preserved specimen is expressed in g.

The specimens used in this redescription were deposited at the Centro Nacional Patagónico (CNP-CONICET), Puerto Madryn, Argentina (CNPICT). Additional specimens were deposited at the Instituto de Biología Marina y Pesquera Almirante Storni (Ichthyological Collection), San Antonio Oeste, Argentina. Specimens of the type series deposited in the Museo Argentino de Ciencias Naturales under number MACN 8806, 8807, 8808 and 8809 were also examined for measurements and counting.

GENETIC STUDY

Specimens of *H. patagonicus* for genetic analysis were obtained from San Antonio Bay during surveys carried out in the period 2006–2009. Tissues samples from tail muscle were obtained from 25 fish, stored in 100% ethanol and dried before DNA extraction. Total DNA was extracted from muscular tissue using a JETQUICK Tissue DNA Spin Kit (GenoMed; www.genomed.com).

The *cytochrome b* gene has been used in a large number of studies and it is a classical tool to elucidate phylogenetic relationships and phylogeographic patterns for the genus *Hippocampus* (Lourie *et al.*, 1999*b*; Jones *et al.*, 2003; Casey *et al.*, 2004; Teske *et al.*, 2004). Primers were designed to amplify the entire *cytochrome b* gene and flanking regions using the complete sequence of the mitochondrial genome of *Hippocampus kuda* Bleeker 1852 (GenBank accession number AP005985; Kawahara *et al.*, 2008), yielding products of *c*.1800 base pairs (bp) (Table I). Amplifications were carried out in a GeneAmp PCR System 2700 (Applied Biosystems; www.appliedbiosystems.com) Thermal Cycler. The polymerase chain reaction (PCR) protocol consisted of an initial cycle of denaturation (94° C for 4 min) followed by 35 cycles of denaturation (94° C for 40 s), annealing (53° C for 1 min) and extension (72° C for 2 min), followed by a final extension step (72° C for 5 min).

Sequencing was attempted using the internal primers designed by Casey *et al.* (2004) and the only acceptable results were achieved using the pairs SHORSES5.3L and SHORSE3.4H. Nucleotide sequences were obtained with an Applied Biosystems 3130*xl* Genetic Analyser. The sequences obtained for eight specimens of *H. patagonicus* were deposited in GenBank under the accession numbers detailed in Table II (*H. patagonicus* 1–8).

To complete the analysis, 25 sequences of different and related species were downloaded from GenBank (accession numbers shown in Table II; www.ncbi.nlm.nih.gov). These included nine different species of seahorses, and *Hippocampus breviceps* Peters 1869, *Hippocampus comes* Cantor 1850 and *Hippocampus coronatus* Temminck & Schelegel 1850 were used as outgroup taxa. Phylogenetic analyses of the molecular data were performed under maximum
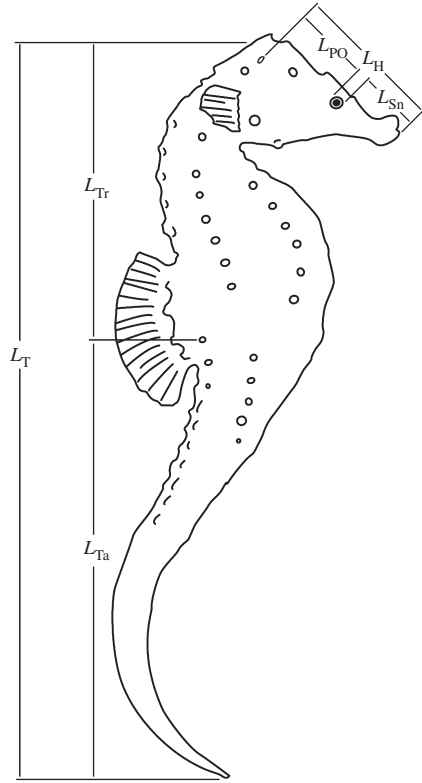
FIG. 2. Morphometric measurements and terms used in this study (illustration represents an adult male of *Hippocampus patagonicus*). Measurements including the tail were taken with the tail straightened. L_{Ta}, tail length; L_{Tr}, trunk length; L_T, total length; L_{PO}, postorbital length; L_H, head length; L_{Sn}, snout length.

parsimony (MP) and Bayesian analysis. For the data set analysed using standard parsimony, the heuristic search procedure consisted of tree bisection and reconnection (TBR) branch swapping applied to a series of 10 000 random addition sequences, retaining 10 trees per replicate, using the programme TNT (Goloboff *et al.*, 2003). No further search strategies were adopted when optimal trees were found for all the replicates. Branch supports were estimated using bootstrap resampling (2000 replications).

The programme ModelTest (Posada & Crandall, 1998) was used to select the most likely model of evolution for the molecular data set. The selected model was incorporated in Bayesian searches for the estimation of phylogenetic relationships. All searches were performed in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). Bayesian searches were run

TABLE I. Seahorse-specific polymerase chain reaction (PCR) and sequencing primers

Primer	Primer sequence (5′–3′)	Priming site
SH CytB F	CAACTTTCAGGGTATGGCTC	−318 to −298
SH CytB R	GCAGTAAGAGCCTACCAATCAA	1527 to 1549
SHORSE5.3L	ATA TCC TTC TGA GGA GCC	412 to 429
SHORSE3.4H	CCA GAT ACA GGT AAA GC	1093 to 1109

TABLE II. The final set of 25 specimen designations, showing the haplotypes that were used in the phylogenetic analysis, the GenBank accession number and the species' known distribution

Species	Haplotype	GenBank accession	Origin
Hippocampus kuda	*Hippocampus kuda2*	AF192685.1	Taiwan
H . kuda	*Hippocampus kuda3*	AF192683.1	Philippines
H . kuda	*Hippocampus kuda1*	AF192687.1	Vietnam
Hippocampus reidi	*Hippocampus reidi1*	AF192694.1	Brazil
H . reidi	*Hippocampus reidi2*	AF192691.1	Caribbean
Hippocampus zosterae	*Hippocampus zosterae1*	AF192706.1	U.S.A.
H . zosterae	*Hippocampus zosterae2*	AF356071.2	Florida (U.S.A.)
Hippocampus hippocampus hippocampus	*Hippocampus hippocampus2*	AF192665.1	U.K.
H . hippocampus hippocampus	*Hippocampus hippocampus1*	AF192666.1	Italy
Hippocampus erectus	*Hippocampus erectus2*	AF192661.1	U.S.A.
H . erectus	*Hippocampus erectus1*	AF192662.1	U.S.A.
H . erectus	*Hippocampus erectus5*	AF356057.1	Virginia (U.S.A.)
Hippocampus patagonicus	*Hippocampus patagonicus MDQ*	EU871944.1	MdP (ARG)
H . patagonicus	*Hippocampus patagonicus SAB*	EU871945.1	SAB (ARG)
H . patagonicus	*Hippocampus patagonicus1*	GQ404494	SAB (ARG)
H . patagonicus	*Hippocampus patagonicus2*	GQ404495	SAB (ARG)
H . patagonicus	*Hippocampus patagonicus3*	GQ404496	SAB (ARG)
H . patagonicus	*Hippocampus patagonicus4*	GQ404497	SAB (ARG)
H . patagonicus	*Hippocampus patagonicus5*	GQ404498	SAB (ARG)
H . patagonicus	*Hippocampus patagonicus6*	GQ404499	SAB (ARG)
H . patagonicus	*Hippocampus patagonicus7*	GQ404500	SAB (ARG)
H . patagonicus	*Hippocampus patagonicus8*	GQ404501	SAB (ARG)
Hippocampus breviceps	*Hippocampus breviceps*	AF192647.1	Australia
Hippocampus coronatus	*Hippocampus coronatus*	AF192658.1	Japan
Hippocampus comes	*Hippocampus comes*	NC_020336.1	Taiwan

MdP (ARG), Mar del Plata, Argentina; SAB (ARG), San Antonio Bay, Argentina.

with four simultaneous chains for 1 000 000 generations, sampling every 100 generations and applying temperatures of 1, 0·5 and 0·3, which influence the rate of switching between chains. The burn-in (*i*.*e*. the generation by which stationarity was reached) was determined by plotting generations *v*. logarithmic likelihood values; all trees below the stationary level were discarded. The remaining trees were used to construct the 50% majority rule consensus tree. In the resulting tree, the posterior Bayesian probabilities (PP) of the internal nodes shared with the MP tree are shown below the branches. The proportion of different nucleotide bases (P-distances) between the holotype of *H* . *patagonicus* and the closest relatives were estimated using MEGA 4.0 (Tamura *et al.*, 2007).

RESULTS

MORPHOMETRIC AND MERISTIC STUDY

Hippocampus patagonicus Piacentino & Luzzatto 2004

Holotype: MACN 8806, 103·6 mm L_T, female. Type locality: 40° 45′ S; 64° 55′ W Argentina, Río Negro, San Antonio Bay. Collected by: unspecified. Date: 10 February 2002.

TABLE III. Meristic and morphometric characteristics from the type series specimens of *Hippocampus patagonicus*

Data	Holotype	Paratypes		
Collection number	MACN 8806	MACN 8807	MACN 8808	MACN 8809
Sex	Female	Male	Female	Male
Total length (mm)	103·6	95·1	103·2	69·1
Trunk length (mm)	33·2	29·1	32·4	22·3
Tail length (mm)	70·4	66	70·8	46·8
Head length (mm)	19·9	18·1	20·1	13·8
Snout length (mm)	7·3	6·2	7·1	4·8
Eye diameter (mm)	3·1	3	3·1	2·5
Number of trunk rings	11	11	11	11
Number of tail rings	37	37	37	37
Number of dorsal fin rays	18	17	18	18
Number of pectoral fin rays	13	14	14	14

Paratypes: MACN 8807, 95·1 mm L_T, male; MACN 8808, 103·2 mm L_T, female; MACN 8809, 69·1 mm L_T, male. Type locality and collection date: the same as the holotype. Collected by: unspecified. Measurements and counts of the type specimens for this study were performed by M. Maggioni (Table III).

Non-type material (catalogued): CNPICT #2003/61, 121·42 mm L_T, female.

Locality: Banco Reparo, San Antonio Bay, San Matías Gulf, 40° 47·200′ S; 64° 54·605′ W, depth: 4 m; collected by: R. González, 22 March 2003. CNPICT #2003/62 (two specimens): (1) 122·65 mm L_T, male; (2) 116·00 mm L_T, female. Same collection date and locality as for the CNPICT #2003/61. Non-type material collected near the type locality. Observations, counting and measurements were performed by R. González (Table IV).

TABLE IV. Meristic and morphometric characteristics from non-type specimens of *Hippocampus patagonicus*

| Data	Non-type specimen		
Collection number	CNPICT #2003/61	CNPICT #2003/62-a	CNPICT #2003/62-b
Sex	Female	Male	Female
Total length (mm)	121·42	122·65	116·00
Trunk length (mm)	38·51	33·25	37·82
Tail length (mm)	81·66	85·16	78·87
Head length (mm)	23·91	21·55	22·62
Snout length (mm)	9·13	7·31	8·06
Eye diameter (mm)	3·19	3·71	3·89
Number trunk rings	11	11	11
Number of tail rings	37	37	37
Number of dorsal fin rays	18	17	18
Number of pectoral fin rays	13	14	13
Mass (g)	6·81	6·47	3·73
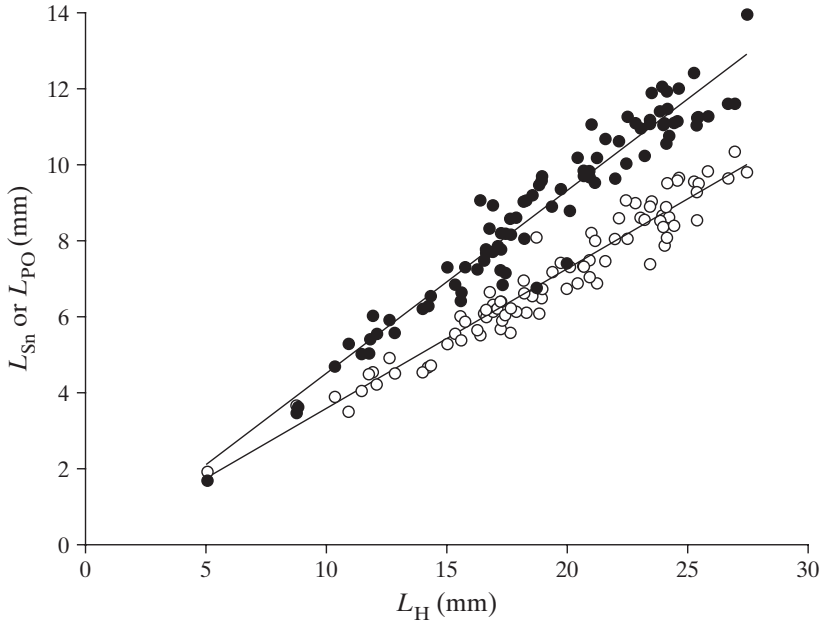
FIG. 3. Relationship between snout length (L_{Sn}; ○) and postorbital length (L_{PO}; ●) with the head length (L_H) for *Hippocampus patagonicus*. The curves were fitted by L_{PO}:L_H, $y = 0.4811x - 0.2954$ ($r^2 = 0.9308$) and L_{Sn}:L_H, $y = 0.3673x - 0.0735$ ($r^2 = 0.9402$).

In addition, a total of 136 specimens (83 females and 53 males) collected between 2001 and 2009 were processed to obtain morphometric and meristic data.

Diagnosis: *H. patagonicus* can be distinguished from *H. erectus*, the only seahorse recorded to date for the Argentine Sea, by the combination of the following morphometric characteristics: (1) in both sexes and all sizes of *H. patagonicus*, the snout length is always less than the postorbital length (Fig. 3), whereas the snout length of *H. erectus* is not shorter than the postorbital length in the largest specimens (Vari, 1982); (2) in both sexes of *H. patagonicus*, the L_{Tr}:L_T is lower than in *H. erectus* (in female *H. patagonicus*: 0.27–0.39, *H. erectus*: 0.36–0.40 and in male *H. patagonicus*: 0.24–0.34, *H. erectus*: 0.33–0.43) and (3) in both sexes of *H. patagonicus*, the L_{Ta}:L_T is higher than in *H. erectus* (0.61–0.78 *v.* 0.54–0.64).

Description: based on 143 specimens (87 females and 56 males) for morphometric characteristics and 48 specimens (26 females and 22 males) for meristic characteristics (21–154 mm L_T); data summarized in Table V. Features in addition to those noted in the diagnosis above are as follows. Skin filaments poorly developed, present in a few younger specimens, usually absent in adults. Coronet small to well developed, with four or five spineless ridges, $I_C = 2$. Cheek spine single and prominent, rounded tips, $I_{CS} = 1–2$. Trunk spines variable in size and shape, prominent for rings seven to 11 on the dorsal, lateral and inferior trunk ridges. Tail spines conspicuous on the first seven rings, $I_S = 2$. Elliptical ventral depression or dark spot present between first and third or fourth tail rings in some adult females.

Colouration: usually drab in life, pale to dark brown with irregular dark striations (Fig. 4). Numerous small, white spots distributed on head and trunk. Spots arranged

TABLE V. Comparison of meristic and morphometric characteristics used in the descriptions of *Hippocampus erectus* and *Hippocampus patagonicus*

	H. erectus (Vari, 1982)	H. erectus (Lourie et al., 1999a)	H. patagonicus (Piacentino & Luzzatto, 2004)	H. patagonicus (this study)
Number of specimens	213	51	22	143
L_T range	30–173 mm	6–19 cm	21–103 mm	21–154 mm
Number of pectoral and tail rings	11 + 33–38	11 + 34–39 (36)	10–11 + 37–41 (38)	11 + 35–38 (37)
Number of subdorsal rings	2–3 + 1–2	2 + 1	2 + 1	2–3 + 1–2
Number of fin rays				
Dorsal	16–20	18–19 (16–20)	16–19 (17)	16–18 (18)
Pectoral	14–17	15–16 (14–18)	12–14 (13)	13–15 (14)
Anal	3–4	–	4	3–4
Head	L_H 0.20–0.27 of L_T; L_{Sn} 0.33–0.46 of L_H or L_{Sn} 2.17–3.03 in L_H	L_{Sn} 0.31–0.45* of L_H or L_{Sn} 2.6 (2.2–3.2) in L_H	L_H 0.1942–0.2897 of L_T; L_{Sn} 0.3437 of L_H or L_{Sn} 2.96 (2.43–3.47) in L_H	L_H 0.16–0.26 of L_T; L_{Sn} 0.32–0.42 of L_H
	D_O 0.12–0.18 of L_H; L_{PO} 0.38–0.49 of L_H; L_{Tr} 0.36–0.40 of L_T (females)	–	–	D_O 0.08–0.29 of L_H; L_{PO} 0.34–0.56 of L_H; L_{Tr} 0.27–0.39 of L_T (females)
Body	L_{Tr} 0.33–0.43 of L_T (males); L_{Ta} 0.54–0.64 of L_T (females); L_{Ta} 0.56–0.67 of L_T (males)	–	L_{Tr} 0.366 of L_T (Ginsburg, 1937). Both sexes.	L_{Tr} 0.24–0.34 of L_T (males); L_{Ta} 0.61–0.72 of L_T (females); L_{Ta} 0.64–0.78 of L_T (males)

L_T, total length; L_{Tr}, trunk length; L_{Ta}, tail length; L_{Sn}, snout length; L_H, head length; L_{PO}, postorbital length; D_O, orbital diameter.
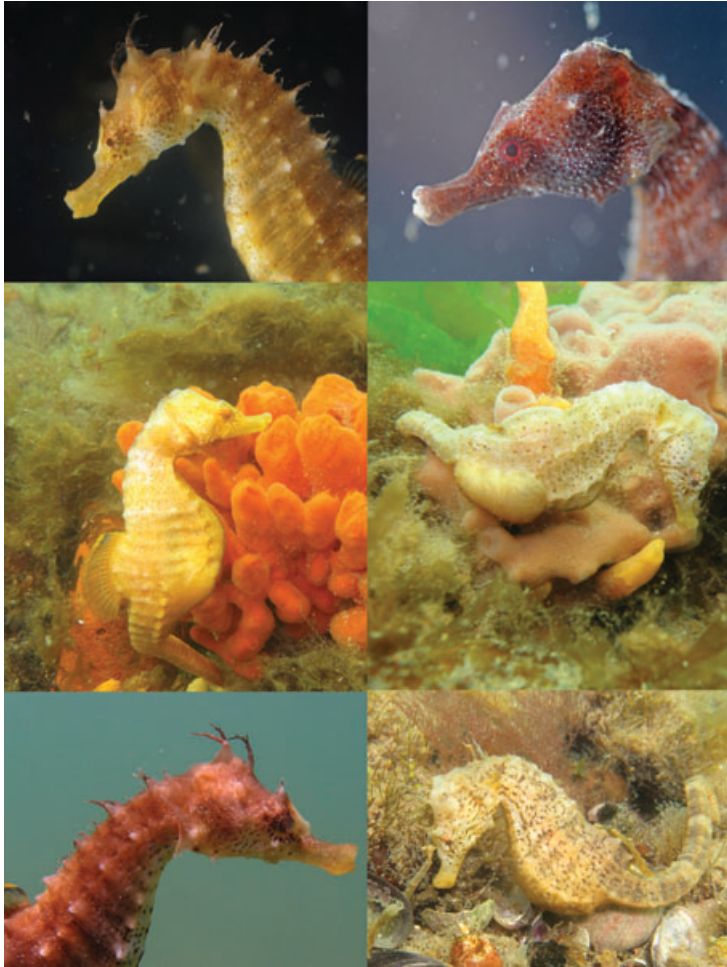*Recalculated from Lourie et al. (1999a).

Fig. 4. Specimens of *Hippocampus patagonicus* in the natural environment, showing variability in colouration, spines and skin filaments.

in a radial pattern around the eye. Other body colourations that are usually observed: black, bright yellow and orange, and occasionally white. Colour does not change when specimens are preserved in alcohol, except for yellow and orange specimens which become pale.

Remarks: *H. patagonicus*, redescribed here, was previously cited by Kuiter (2003) as an undetermined or undescribed species from San Antonio Bay (Patagonia, Argentina), referring to it as Patagonian seahorse [*Hippocampus* cf. *hippocampus* (L. 1758)]. *Hippocampus patagonicus* feeds mainly on amphipods (Gammaridae and Caprellidae), juveniles of brachyuran decapods (Grapsidae) and carideans (Storero & González, 2008). Its life span reaches 2 years and its growth rates are similar to those reported for other species of *Hippocampus* (R. González, unpubl. data). Its known parasite species include cystacanths of the acanthocephalan *Corynosoma australe* (Braicovich *et al.*, 2005). Other studies performed on this species also
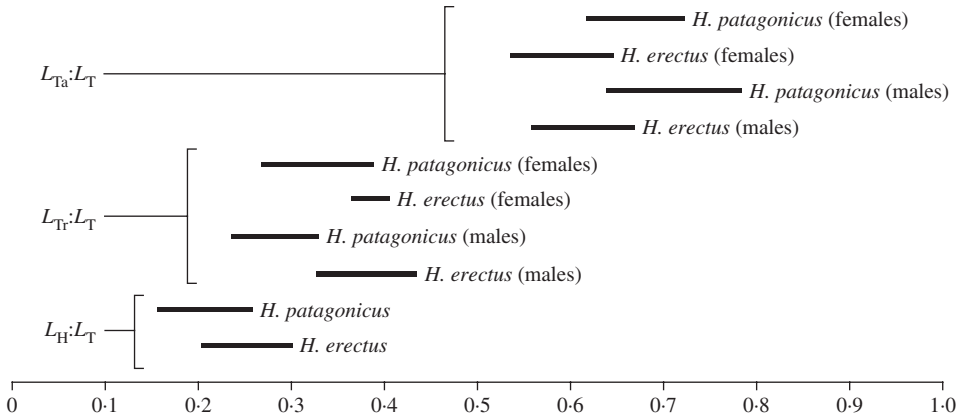
FIG. 5. Ranges of diagnostic morphometric characteristics (see Fig. 2) reported for *Hippocampus erectus* and *Hippocampus patagonicus*. Data for *H. erectus* taken from Vari (1982) and Lourie et al. (1999a).

include aquarium rearing and prey selection in captivity (Storero & González, 2009).

Distribution: *H. patagonicus* is very conspicuous in San Antonio Bay during spring and summer when densities range from 0·3 to 1·5 individuals per 10 m^2 (R. González, unpubl. data). The main areas where this species is found in San Antonio Bay are indicated in Fig. 1. It is found mainly on soft bottoms covered by rodophyte and chlorophyte algae at depths of 1–7 m (low tide). Isolated individuals occasionally occur deeper on bivalve fishing grounds (*e.g.* oyster, mussel and scallops) outside San Antonio Bay, in the north-western part of the San Matías Gulf. Although a significant number of research and fishing surveys have been carried out during the last two decades in all the areas of the San Matías Gulf, *H. patagonicus* has been mainly recorded in San Antonio Bay and its neighbouring areas.

Comparison: comparisons of meristic and morphometric characteristics were made in relation to the type specimens reported in the original description (Piacentino & Luzzatto, 2004) and for *H. erectus*, another species previously cited from Uruguayan and Argentine waters, in the south-west Atlantic biome. Because specimens of *H. erectus* were not available in Argentine collections and raw data were not reported in their existing descriptions, the comparison was made using the ranges of the measurements of the diagnostic characteristics reported by Vari (1982). A significant difference between adults of *H. patagonicus and H. erectus* was observed in the $L_{Sn}:L_H$ (Fig. 3), and the range of overlap between both species was low or null in the relationships $L_{Ta}:L_T$ (14–17%) and $L_{Tr}:L_T$ (0–23%) for both sexes (Fig. 5).

All the meristic and morphometric characteristics reported for the holotype and paratypes of *H. patagonicus* in the original description by Piacentino & Luzzatto (2004) agree with values and ranges observed in this study. The main taxonomic characteristics from previous descriptions of *H. erectus* (Vari, 1982; Lourie *et al.*, 1999a), those characteristics reported from the original description of *H. patagonicus* and those from this study are summarized in Table V. The comparison with data from the original description of *H. patagonicus* show that, with the exception of the number of the pectoral fin rays, all the meristic and morphometric measurements
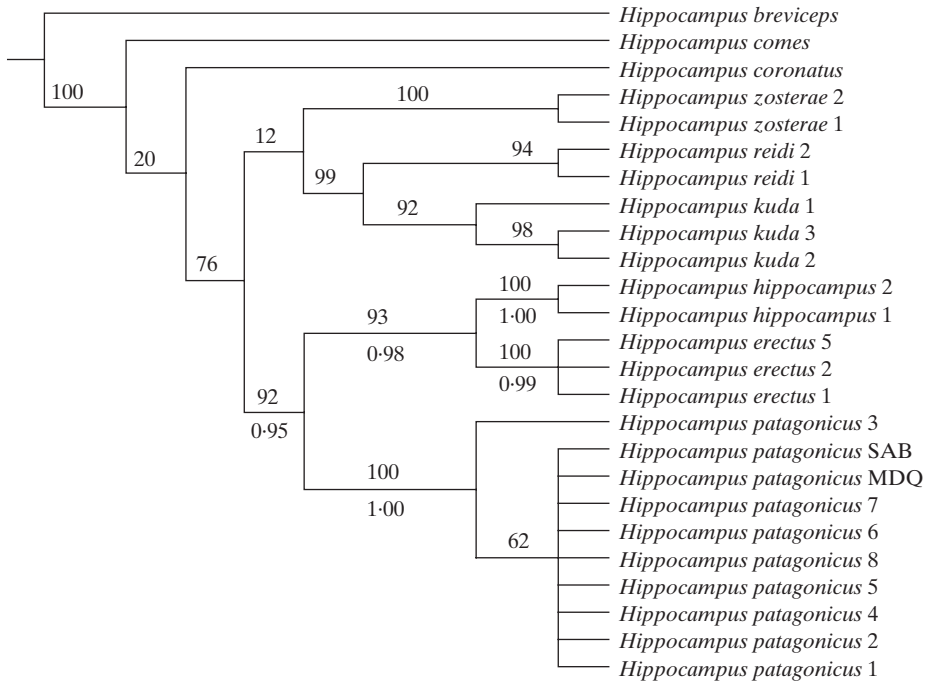
FIG. 6. Maximum parsimony tree. Numbers above branches show bootstrap values. Numbers under the branches represent posterior Bayesian probabilities (PP) higher than 50% for *Hippocampus patagonicus* group and its closest relatives.

used as diagnostic characteristics by Piacentino & Luzzatto (2004) are not significantly different from those of *H. erectus* (Table V) and do not allow the accurate differentiation between both species. On the contrary, only the distinctive characteristics identified in this study [noted in the *Diagnosis* as (1), (2) and (3)], not analysed by Piacentino & Luzzatto (2004), allow a better diagnosis of *H. patagonicus*.

GENETIC STUDY

The standard parsimony analysis of the aligned *cytochrome b* sequences (578 characteristics, 139 parsimony-informative) yielded one most parsimonious tree of score 389 (Fig. 6). The phylogenetic relationships show that specimens of *H. patagonicus* appear as a natural group. Also, this monophyletic cluster emerges as a sister group to the one comprising *H. erectus* and *H. hippocampus*. These relationships show high bootstrap support values for every node. The model selected by Model-Test (GTR + I + G) (Rodríguez *et al.*, 1990) was incorporated in Bayesian searches. The same relationships for these groups emerged in most of the trees visited during the stationary period of the Metropolis Coupled Markov Chain Monte Carlo (MCMCMC), with high posterior probabilities, in concordance and supporting the parsimony analysis. These posterior probabilities (PP) are also indicated in Fig. 6. The P-distances obtained for *H. patagonicus* and its closest relatives are shown in Table VI. Variance for P-distances was calculated by bootstrap method.

TABLE VI. P-distances within *Hippocampus patagonicus* and with the closest relatives (s.e. < 5% of the values)

	H. patagonicus	*H. erectus*	*H. hippocampus*	All taxa*
H. patagonicus	0·0013			
Hippocampus erectus	0·0588	0·0049		
Hippocampus hippocampus	0·0783		0·0035	
All taxa*	0·1372			0·1217

*Includes the eight species *Hippocampus* used in the analysis.

DISCUSSION

Only three species *Hippocampus* have been previously cited for the south-west Atlantic Ocean: *Hippocampus reidi* Ginsburg 1933, *H. erectus* (Vari, 1982; Lourie *et al.*, 1999*a*) and *H. patagonicus* (Piacentino & Luzzatto, 2004). Lourie *et al.* (1999*a*) refer to specimens collected north of La Plata River (36° S), whereas Pozzi & Bordale (1935) reported *H. punctulatus* (= *H. erectus*) off Patagonia at 43° 30′ S, which is the only record south of 36° S, but unfortunately, specimens were not preserved.

As mentioned by several authors (Casey *et al.*, 2004; Foster & Vincent, 2004), morphological examination alone might be inadequate for revising the taxonomy of seahorses, especially given the extent of intraspecific phenotypic variation and the potential for cryptic or sibling species. In this respect, the results presented here show that molecular markers are a useful tool to help discriminate between closely related, often cryptic, species of seahorses and prove the valid designation of *H. patagonicus*.

Vari (1982) and Lourie *et al.* (1999*b*) mentioned variations in colour pattern, skin filament development, coronet shape and number and size of tubercles and spines on the head and body, in relation to ontogeny, sex and geographic distribution for *H. erectus*. Curtis (2006) concluded that the presence or absence of skin filaments is an unreliable characteristic to identify European seahorses, as growth of skin filaments coincides with maturation in *Hippocampus guttulatus* Cuvier 1829 and may be highly variable in *H. hippocampus*. In this study, significant variations in colouration, spines and presence and size of skin filaments were recorded in *H. patagonicus* from San Antonio Bay. Owing to this, all these features are considered unsuitable as diagnostic characteristics.

The set of morphometric and meristic characteristics and morphological features used prior to this study to describe *H. patagonicus* are insufficient for an unambiguous identification of this species. Moreover, the low number of specimens analysed and the narrow range of sizes do not allow a sufficient analysis when ontogenetic variation in morphometric relationships is considered. For example, there is a considerable variation in snout length:head length and postorbital length:head length. In contrast, the results presented here expand the number of specimens and the data set, providing a statistically supported conclusion to confirm the taxonomic identity of *H. patagonicus* through genetic testing. The differences detected in this study were perceptible only when comparing specific morphometric relationships from large (and broad size range) samples of fishes; therefore,

genetic analysis appears as a necessary tool to unequivocally differentiate these species.

The *cytochrome b* mitochondrial gene proved to be appropriate to resolve conflicts at species level as demonstrated in previous studies (Casey *et al.*, 2004; Teske *et al.*, 2004). The parsimony search showed only one fully resolved tree with bootstrap values near 100% for several groups. In addition, the same relationships among species were recovered in the Bayesian analysis. P-distance analysis showed that the distances within species are all in the same range, and values for *H. patagonicus* and its closest relatives are significantly different. These results clearly demonstrate the high level of confidence of the analyses made, and strongly support the morphometric evidence. Both phylogenetic analyses reinforce the hypothesis that *H. patagonicus* is a distinct species, and supports the phylogeny shown in Teske *et al.* (2004) in which *H. erectus* and *H. hippocampus* were also recovered as sister taxa in the context of the Atlantic Ocean biome. Luzzatto *et al.* (2012) suggested the presence of *H. patagonicus* in southern Brazil, highlighting the need to resolve the taxonomic status of *H. erectus* in the south-western Atlantic Ocean and the validation of the name *H. patagonicus*, as has been done in this study. On the other hand, Boehm *et al.* (2013) studied how marine barriers shaped the demographic history of Atlantic Ocean seahorses. Those authors suggested that while rafting could have been the mechanism for range expansion over large distances, it might not have been sufficient for sustaining genetic flow across major barriers, resulting in lineage divergence.

In conclusion, this study provides a redescription and the validation of *H. patagonicus* based on unpublished morphometric data and genetic evidence, which has included the analysis of specimens of the type series and numerous samples of fish collected at the type locality. It corroborates the results of other studies and definitively clarifies the taxonomic status of *H. patagonicus*. The taxonomy of seahorses has been the subject of much controversy during recent years; therefore, studies such as the present, which promote the clear understanding of species identification, are of major importance in view of the growing interest in the international conservation and management of seahorses (Vincent *et al.*, 2011).

References

Boehm, J., Woodall, L., Teske, P., Lourie, S., Baldwin, C., Waldman, J. & Hickerson, M. (2013). Marine dispersal and barriers drive Atlantic seahorse diversification. *Journal of Biogeography* **40,** 1839–1849. doi: 10.1111/jbi.12127

Braicovich, P., González, R. & Tanzola, D. (2005). First record of *Corynosoma australe* (Acanthocephala: Polymorphidae) parasitizing seahorse *Hippocampus* sp. (Pisces: Syngnathidae) in Patagonia. *Acta Parasitologica* **50,** 145–149.

Casey, S., Hall, H., Stanley, H. & Vincent, A. (2004). The origin and evolution of seahorses (genus *Hippocampus*): a phylogenetic study using the cytochrome *b* gene of mitochondrial DNA. *Molecular Phylogenetics and Evolution* **30,** 261–272.

Curtis, J. M. R. (2006). A case of mistaken identity: skin filaments are unreliable for identifying *Hippocampus guttulatus* and *Hippocampus hippocampus*. *Journal of Fish Biology* **69,** 1855–1859.

Foster, S. & Vincent, A. (2004). Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology* **65,** 1–61.

Ginsburg, I. (1937). Review of the seahorse (Hippocampus) found on the coast of the American continents and of Europe. *Proceedings of the United States National Museum* **83,** 497–594.

Huelsenbeck, J. & Ronquist, F. (2001). MrBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17,** 754–755.

Jones, A. G., Moore, G. I., Kvarnemo, C., Walker, D. & Avise, J. (2003). Sympatric speciation as a consequence of male pregnancy in seahorses. *Proceedings of the National Academy of Sciences of the United States of America* **100,** 6598–6603.

Kawahara, R., Miya, M., Mabuchi, K., Lavoué, S., Inoue, J., Satoh, T., Kawaguchi, A. & Nishida, M. (2008). Interrelationships of the 11 gasterosteiform families (sticklebacks, pipefishes, and their relatives): a new perspective based on whole mitogenome sequences from 75 higher teleosts. *Molecular Phylogenetics and Evolution* **46,** 224–236.

Kuiter, R. (2001). Revision of the Australian seahorses of the genus *Hippocampus* (Syngnathiformes: Syngnathidae) with descriptions of nine new species. *Records of the Australian Museum* **53,** 293–340.

Lourie, S., Vincent, A. & Hall, H. (1999*a*). *Seahorses: An Identification Guide to the World's Species and their Conservation*. London: Project Seahorse.

Lourie, S., Pritchard, J., Casey, S., Truong, S., Hall, H. & Vincent, A. (1999*b*). The taxonomy of Vietnam's exploited seahorses. *Biological Journal of the Linnean Society* **66,** 231–256.

Luzzatto, D., Sieira, R., Pujol, M. & Díaz de Astarloa, J. (2012). The presence of the seahorse *Hippocampus patagonicus* in the Argentine Sea based on the cytochrome b sequence of mitochondrial DNA. *Cybium* **36,** 329–333.

Menni, R., Ringuelet, R. & Aramburu, R. (1984). *Peces Marinos de la Argentina y Uruguay. Reseña Histórica. Claves de familias, géneros y especies. Catálogo crítico*. Buenos Aires: Ed. Hemisferio Sur S.A.

Piacentino, G. & Luzzatto, D. (2004). *Hippocampus patagonicus* sp. nov., nuevo caballito de mar para a Argentina (Pisces, Syngnathiformes). *Revista del Museo Argentino de Ciencias Naturales*, n.s. **6**, 339–349.

Piola, A. & Scasso, L. (1988). Circulación en el Golfo San Matías. *Geoacta* **15,** 33–51.

Posada, D. & Crandall, K. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14,** 917–918.

Pozzi, A. & Bordale, L. (1935). Cuadro sistemático de los peces marinos de la República Argentina. *Anales de la Sociedad Científica Argentina Buenos Aires* **120,** 145–189.

Rodríguez, F., Oliver, J., Marín, A. & Medina, J. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142,** 485–501.

Schnack, E., Aliotta, S., Isla, F. & Lizasoain, G. (1996). Formas de fondo asociadas a un delta de reflujo macromareal, San Antonio Oeste, Provincia de Río Negro, Argentina. *Thalassas* **12,** 121–135.

Storero, L. & González, R. (2008). Feeding habits of the seahorse *Hippocampus patagonicus* in San Antonio Bay (Patagonia, Argentina). *Journal of the Marine Biological Association of the United Kingdom* **88,** 1503–1508.

Storero, L. & González, R. (2009). Prey selectivity and trophic behaviour of Patagonian seahorse *Hippocampus patagonicus* in captivity. *Journal of the World Aquaculture Society* **40,** 394–401.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24,** 1596–1599.

Teske, P., Cherry, M. & Conrad, M. (2004). The evolutionary history of seahorses (Syngnathidae: Hippocampus): molecular data suggest a West Pacific origin and two invasions of the Atlantic Ocean. *Molecular Phylogenetics and Evolution* **30,** 273–286.

Vari, R. (1982). The seahorses (Subfam. Hippocampinae). In *Fishes of Western North Atlantic* Part 8 (Bolhke, J., Cohen, D., Collette, B., Eschmeyer, W., Gibbs, R., Pietsch, T. Jr., Richards, W., Smith, C. & Thompson, K., eds), pp. 173–189. New Haven, CT: Memoir Sears Foundation for Marine Research.

Vincent, A., Foster, S. & Koldewey, H. (2011). Conservation and management of seahorses and other Syngnathidae. *Journal of Fish Biology* **78,** 1681–1724.

Wilson, M. & Vincent, A. (1999). Preliminary success in closing the life cycle of exploited seahorse species *Hippocampus* spp. in captivity. *Aquarium Sciences and Conservation* **2,** 179–196.

Electronic References

Goloboff, P., Farris, J. & Nixon, K. (2003). *T.N.T. Tree Analysis using New Technology.* Available at http://www.zmuc.dk/public/phylogeny/tnt/ (last accessed June 2009).

IUCN (2013). *IUCN Red List of Threatened Species. Version 2012.2.* Available from http://www.iucnredlist.org/ (last accessed February 2013).

Kuiter, R. (2003). *Seahorses, Seadragons, Pipehorses, Pipefishes and their Relatives. Pictorial Guide to Singnathiformes* (Kuiter, R.H. & Chettle, M., eds). Available as a CD/ROM from Zoonetics, Seaford, Australia.