First records of Hippocampus algiricus in the Canary Islands (north-east Atlantic Ocean) with an observation of hybridization with Hippocampus hippocampus

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BRIEF COMMUNICATION

First records of *Hippocampus algiricus* in the Canary Islands (north-east Atlantic Ocean) with an observation of hybridization with *Hippocampus hippocampus*

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Morphometric and genetic analyses confirmed the first records of the West African seahorse *Hippocampus algiricus* at Gran Canaria Island (north-east Atlantic Ocean), and also the first evidence of interspecific hybridization in seahorses. These results provide additional data on the distribution of *H. algiricus* that may help to establish future conservation strategies, and uncover a new potential sympatric scenario between *H. algiricus* and *Hippocampus hippocampus*.

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Despite the huge conservation interest, scarce information is available on population distribution limits and interspecific relationships for most seahorse species. To date, the European short-snouted seahorse *Hippocampus hippocampus* (L. 1758) is the only seahorse species reported in the Canary Islands (López *et al.*, 2010). During seahorse population assessments conducted at Gran Canaria during 2008–2009, however, two male specimens with morphometric characteristics inconsistent with *H. hippocampus* (Lourie *et al.*, 2004) were sighted in Melenara Bay over a seagrass bed [Fig. 1(a)]. The specimens, coded GC1 and GC2, showed rounded coronets overhanging at the back of their head [Fig. 1(b)]. Morphological data obtained *in situ* from live specimens suggests their classification as the West African seahorse *Hippocampus algiricus* Kaup 1856, a little known species previously reported as distributed along the West African Atlantic Ocean coasts (Lourie *et al.*, 2004), but never sighted at the Canary Islands, nor at latitudes north of Senegal (Czembor, 2012). With the appropriate permission from

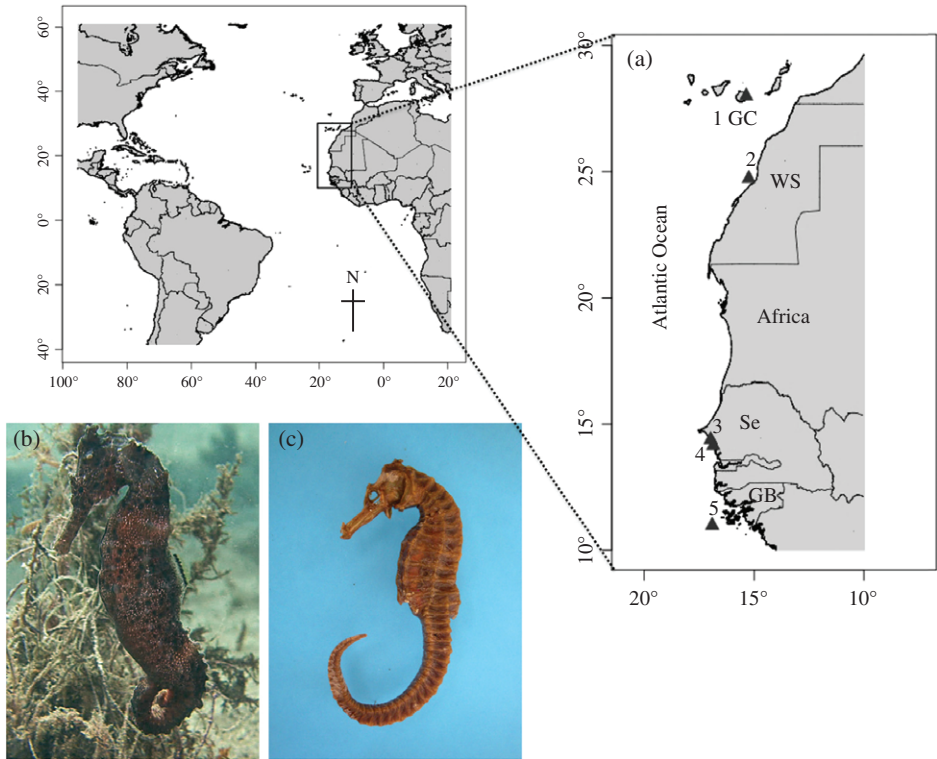†Author to whom correspondence should be addressed. Tel.: +34 928132900; email: francesco_25@hotmail.com

FIG. 1. (a) Location of sampling sites used in this study: 1, Gran Canaria (27° 59′ N; 15° 22′ W; Spain; GC), 2, Western Sahara (24° 44′ N; 15° 15′ W; WS), 3, Mbour (14° 24′ N; 16° 58′ W; Senegal, Se), 4, Joal Fadiouth (14° 10′ N; 16° 51′ W; Senegal, Se) and 5, Guinea-Bissau (10° 59′ N;16° 54′ W, GB). (b) Unidentified seahorse specimen, GC2. (c) Holotype of *Hippocampus algiricus* (MNHN-0000-6084, Algeria; Museum national d'Histoire Naturelle, Paris, France).

regional authorities, the seahorse GC2 was captured and maintained at the University of Las Palmas, Spain. Pregnant upon capture, it released 780 newborn seahorses after a period of 12 days.

To test the hypothesis that GC1 and GC2 were two different specimens of *H. algiricus*, morphological and genetic analyses were conducted against reference samples of *H. hippocampus* and *H. algiricus* collected from several north-east Atlantic Ocean coasts. These analyses also included GC2's offspring to confirm the known paternity and to obtain genetic inferences about the unknown maternity.

The male GC2 died of a tail infection 1 month after capture and was dried for morphological comparisons with other dried specimens, to complement the scarce data available for *H. algiricus* [a single holotype reference was available at the Museum National d'Histoire Naturelle, Paris, France; Fig. 1(c)]. Offspring were preserved for biometric analysis [20 samples in 10% buffered formalin (Panreac T-143091; www.panreac.es)] and genetic analysis (seven samples in 100% ethanol; IDGC2.1-7). The remaining offspring were placed in aquaria for further studies.

Available dried reference samples of *H. hippocampus* and *H. algiricus* were obtained from the edges of their geographic range distribution on north-east Atlantic Ocean

coasts [Lourie *et al.*, 2004; Fig. 1(a)]: (1) *H. hippocampus* Gran Canaria (GC3–GC7), Western Sahara (WS1 and WS2), Senegal (Se1 and Se2 from Joal-Fadiouth; Se4 from Mbour) and (2) *H. algiricus* Senegal-Mbour (Se3) and Guinea-Bissau (GB1 and GB2).

Proportional measurements and counts based on 14 morphometric and seven meristic variables (Table I) were undertaken on dried seahorses. Statistical analyses between species were performed using *t*-tests (Zar, 2009). Results were analysed using the SPSS Statistical Package (www-01.ibm.com/support). Reference specimens of both species show significant differences (*t*-test, $P < 0.05$) in proportional measurements related to head and trunk, which tend to reflect variations in morphological shape: specimens of *H. algiricus* and GC2 show significantly longer and thinner snouts and heads than specimens of *H. hippocampus* (Table I). GC2, similar to the references of *H. algiricus*, also shows more pectoral-fin rays than *H. hippocampus*. In contrast, GC2 and specimens of *H. hippocampus* and *H. algiricus* share a number of morphometric characteristics and meristic counts. Thus, the number of trunk rings (11), anal-fin rays (4), cheek (2) and eye (2) spines is identical in all specimens evaluated.

Subsequently, a principal co-ordinate analysis (PCoA; Gower, 1966) based on proportional measurements and counts (Table I) was produced (PRIMER-E Ltd; www.primer-e.com). The ordination diagram grouped GC2 with specimens of *H. algiricus*, clearly distant from the group of *H. hippocampus* (Fig. 2). After morphological study, the GC2 specimen is now deposited in the vertebrates collection of Museo de Ciencias Naturales (Tenerife, Spain), catalogue number TFMCB-MVP/001918, and the dried reference *H. algiricus* and *H. hippocampus* specimens in the collection of the GIA research group (Grupo de Investigación en Acuicultura) at the University of Las Palmas.

Measurements of biometric and meristic variables were performed on offspring using a profile projector (Mitutoyo PJ-A3000; http://wikimachine.com/) and a binocular microscope (Leica M-125; www.leica-microsystems.com), respectively (Lourie, 2003). The biometric variables obtained were snout (mean $\pm$ s.d. $= 0.94 \pm 0.08$ mm), head (mean $\pm$ s.d. $= 2.14 \pm 0.09$ mm), trunk (mean $\pm$ s.d. $= 2.99 \pm 0.14$ mm), tail (mean $\pm$ s.d. $= 5.36 \pm 0.34$ mm) and standard length (L_S) (mean $\pm$ s.d. $= 10.49 \pm 0.48$ mm). For meristic variables, values registered were the number of dorsal (18), anal (4) and pectoral (15 or 16) fin rays, and trunk (11) rings.

At birth, offspring of GC2 showed biometric values within the same range as those reported for *H. hippocampus* (Otero-Ferrer, 2012). For meristic variables, however, offspring showed pectoral-fin ray counts within the reported range for *H. algiricus* (Lourie *et al.*, 2004), but higher than the ranges previously described on offspring of *H. hippocampus* (Otero-Ferrer, 2012).

Genetic analyses were performed to confirm the morphological identification of the GC1 and GC2 specimens, and also of GC2's offspring. Dorsal-fin samples were collected *in situ* from live GC1 and GC2 specimens for DNA isolation (NucleoSpin Tissue XS, Macherey-Nagel; www.mn-net.com). Tail samples (*c*. 5 mm long) from dried reference specimens were hydrated for DNA purification [NucleoSpin Tissue columns, Macherey-Nagel; Sanders *et al.* (2008)], providing high quality DNA for six specimens (GC3–GC5, WS1, Se1 and Se3). Chelex resin (www.bio-rad.com) was used for extracting DNA from the offspring (IDGC2) (Estoup *et al.*, 1996).

For all samples, a 1023 bp fragment of the mitochondrial cytochrome *b* (*cytb*) gene was amplified and sequenced using the protocols described by López *et al.* (2010) and the following primers: forward L14724 (Irwin *et al.*, 1991; redesigned in this

TABLE I. Morphometric measurements and comparative counts of meristic variables used in principal co-ordinate analysis of *Hippocampus* spp. specimens studied [sample code in the first column (see footnote) and species identification are shown]. Measurements are expressed as percentages of standard length (L_S), trunk length (TrL), head length (HL) or snout length (SnL) compared with other morphometric measurements

Code	Species ID	Proportional measurements															Counts		
		HL:L_S	TrL:L_S	TL:L_S	HD:L_S	SnD:SnL	OD:HL	PO:HL	HL:SnL	CH:HL	TD9:L_S	DL:L_S	PL:L_S	TD4:L_S	TD4:TrL	TD9:TrL	Tail	Dorsal	Pectoral
GC2	Unidentified	0·19	0·27	0·54	0·46	0·22	0·17	0·34	2·17	0·27	0·10	0·07	0·03	0·06	0·23	0·36	35	18	17
GB1	*H. algiricus*	0·18	0·28	0·53	0·45	0·24	0·16	0·32	2·18	0·27	0·10	0·06	0·03	0·06	0·21	0·36	35	17	17
GB2	*H. algiricus*	0·17	0·28	0·55	0·48	0·23	0·16	0·35	2·21	0·28	0·11	0·07	0·03	0·06	0·23	0·41	35	18	17
Se3	*H. algiricus*	0·18	0·27	0·55	0·49	0·26	0·16	0·41	2·33	0·29	0·10	0·07	0·03	0·06	0·23	0·38	35	17	17
GC3	*H. hippocampus*	0·18	0·29	0·53	0·57	0·32	0·19	0·40	2·61	0·34	0·10	0·07	0·03	0·07	0·24	0·33	36	17	14
GC4	*H. hippocampus*	0·20	0·31	0·50	0·52	0·31	0·19	0·36	2·61	0·33	0·10	0·06	0·04	0·07	0·24	0·32	36	18	14
GC5	*H. hippocampus*	0·21	0·31	0·48	0·53	0·31	0·18	0·37	2·55	0·33	0·08	0·06	0·04	0·07	0·24	0·27	35	17	14
GC6	*H. hippocampus*	0·18	0·28	0·54	0·56	0·31	0·20	0·38	2·58	0·43	0·09	0·08	0·03	0·06	0·22	0·33	36	17	14
GC7	*H. hippocampus*	0·20	0·30	0·50	0·50	0·35	0·19	0·43	2·72	0·43	0·10	0·05	0·05	0·06	0·21	0·36	36	17	14
WS1	*H. hippocampus*	0·17	0·27	0·56	0·64	0·37	0·17	0·43	3·04	0·43	0·11	0·06	0·03	0·08	0·29	0·40	37	17	14
WS2	*H. hippocampus*	0·15	0·28	0·57	0·62	0·40	0·17	0·44	3·02	0·42	0·12	0·07	0·03	0·08	0·28	0·41	36	17	14
Se1	*H. hippocampus*	0·18	0·30	0·53	0·55	0·31	0·15	0·40	2·50	0·41	0·11	0·05	0·03	0·08	0·26	0·39	36	17	14
Se2	*H. hippocampus*	0·17	0·29	0·54	0·56	0·32	0·15	0·44	2·80	0·38	0·11	0·08	0·03	0·07	0·25	0·38	36	15	14
Se4	*H. hippocampus*	0·17	0·31	0·53	0·61	0·32	0·15	0·44	2·72	0·37	0·12	0·07	0·03	0·08	0·26	0·40	36	17	14
	t-test	*	*	***	***	***		*	***	***				**					

SnD, snout depth; HD, head depth; OD, orbital depth; CH, coronet height; DL, dorsal-fin base length; PL, pectoral-fin base length; PO, post-orbital length; TD4, trunk depth between fourth and fifth rings; TD9, trunk depth between ninth and 10th rings (Lourie, 2003). Meristic counts are tail rings, dorsal-fin rays and pectoral fin rays (Lourie, 2003). GC2, unidentified *Hippocampus* sp. captured in Gran Canaria (Spain); GB1–2, *Hippocampus algiricus* from Guinea-Bissau; Se3, *H. algiricus* from Senegal; GC 3–7, *Hippocampus hippocampus* from Gran Canaria (Spain); WS1–2, *H. hippocampus* from Western Sahara coast; Se1, 2, 4, *H. hippocampus* from Senegal. Asterisks indicate significant differences in comparisons between specimens identified as *H. algiricus* (including GC2) and *H. hippocampus* (two-tailed *t*-test, $*P < 0.05$, $**P < 0.005$, $***P < 0.001$).
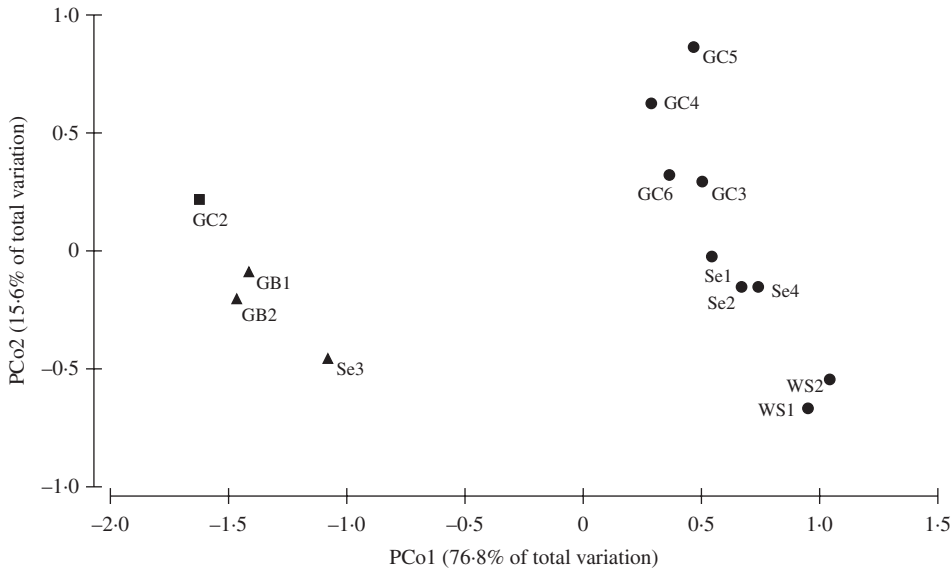
FIG. 2. Ordination plot using the first and second axes of a principal co-ordinate analysis for *Hippocampus* spp. samples in this study based upon morphometric and meristic variables combined using Bray–Curtis index of similarity. The plot includes the undetermined specimen GC2 from Gran Canaria (■) and several morphological reference samples of *Hippocampus algiricus* (▲) and *Hippocampus hippocampus* (●), determined according to criteria established by Lourie *et al.* (2004). Geographical sample codes are from Fig. 1.

study: 5′-GCTTGAAAAACCACCGTTGTA-3′), reverse SHORSE3.4H and internal SHORSE5.3L (Casey *et al.*, 2004). All *cytb* sequences were aligned (CLUSTAL-X 1.8; Thompson *et al.*, 1997), including published haplotypes for reference seahorse species (Casey *et al.*, 2004; Fig. 3). GC1 and GC2 specimens showed the same novel *cytb* haplotype, which clustered in the neighbour-joining tree (MEGA 4.02; Tamura *et al.*, 2007) with sequences of *H. algiricus* from Ghana (Casey *et al.*, 2004) and Senegal (this study), but separated from the *H. hippocampus* clade (Fig. 3) concordant with morphology (Fig. 2). Interestingly, the *cytb* sequence observed for all IDGC2 offspring clusters with haplotypes of *H. hippocampus*, including five novel sequences (*cytb.Hhip*1−5) detected in this study from Western Sahara, Senegal and Gran Canaria. Given the maternal inheritance of mitochondrial DNA, it suggests an interspecific hybridization event of GC2 with a female *H. hippocampus* (Fig. 3). Offspring haplotype (*cytb.Hhip*6) groups with Gran Canaria sequences and one coincides with a common haplotype across the species' range (*cytb.Hhip*3 = DQ288340; Woodall *et al.*, 2011). Novel haplotypes from this study were submitted to GenBank (JX467645−JX467651 and KF650988).

Given the absence of microsatellite markers for *H. algiricus*, 10 loci used in *H. hippocampus* were tested on GC1, GC2 and IDGC2 samples, as well as on West African specimens of *H. algiricus* (Se3) and *H. hippocampus* (WS1 and Se1), following the conditions reported by López *et al.* (2010). Seven loci are informative (Table II), and the rest showed amplification failure (*Hgu*-USC12) or monomorphism (*Hgu*-USC2 and *Habd*3) in *H. algiricus*, expected outcomes for interspecific microsatellite cross-amplification (López *et al.*, 2010). GC1, GC2 and the specimen of
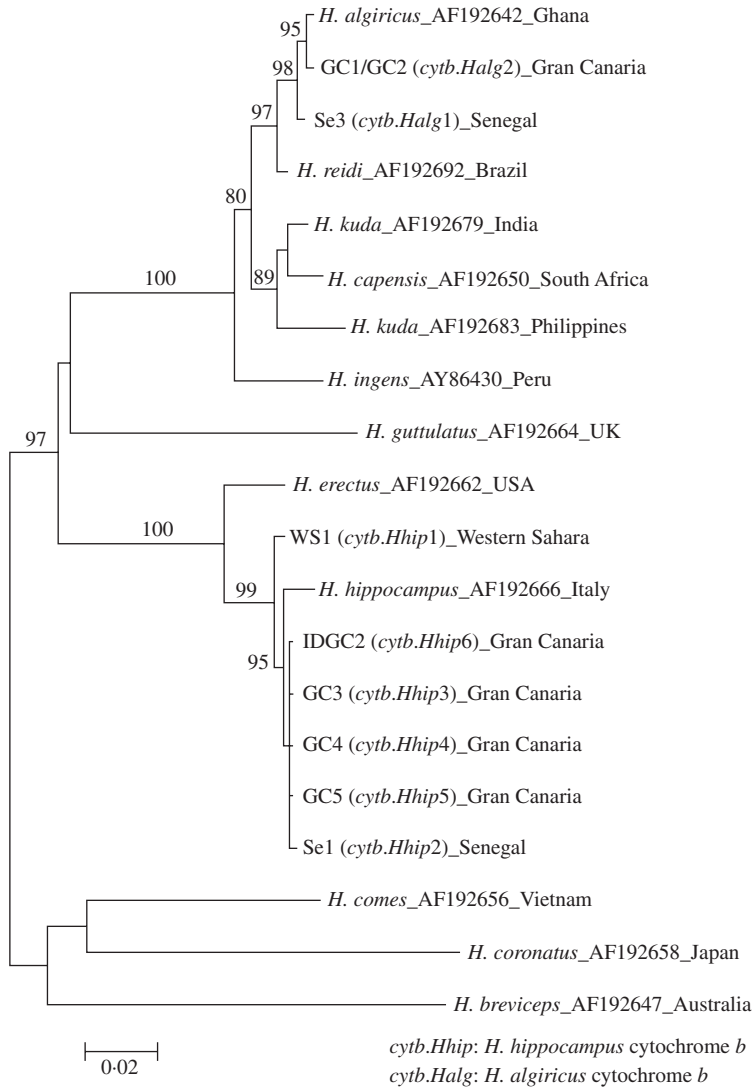
Fig. 3. Neighbour-joining (NJ) phylogenetic analysis for cytochrome *b* (*cytb*) haplotypes of GC1 and GC2 specimens and GC2's offspring (IDGC2) with reference *Hippocampus hippocampus* (*cytb.Hhip*) and *Hippocampus algiricus* (*cytb.Halg*) haplotypes from this study and those from Casey *et al.* (2004): the *kuda* complex (*H. algiricus*, *Hippocampus reidi*, *Hippocampus capensis*, *Hippocampus kuda* and *Hippocampus ingens*), Atlantic (*H. hippocampus*, *Hippocampus guttulatus* and *Hippocampus erectus*) and outgroup species (*Hippocampus comes*, *Hippocampus breviceps* and *Hippocampus coronatus*). Well-supported nodes are indicated with bootstrap values given as percentage (NJ ≥ 75%).

H. algiricus from Senegal (Se3) show atypical peak shapes and allelic sizes at most loci (*Hgu*-USC4, 9, 13, *Hca*μ27, 33) against data of *H. hippocampus* from Gran Canaria (López *et al.*, 2010) and West Africa, supporting mitochondrial and morphological results. Despite sampling size limitations, the microsatellite loci provided conclusive results on the individual identity of GC1 and GC2 (Table II), temporal resampling of

TABLE II. Genotypes at seven informative microsatellite loci for the two unidentified seahorses sampled in Gran Canaria Island, Spain, GC1 and GC2. Genotypes for the reference *Hippocampus algiricus* from Senegal (*Halg*; Se3) together with the allelic ranges of *Hippocampus hippocampus* (*Hhip*) from Melenara population (Gran Canaria; López *et al.*, 2010) and West African coast (WS1, Se1) are also shown for comparison

	Hgu-USC4	Hgu-USC5	Hgu-USC9	Hgu-USC13	Habd9	Hcaμ27	Hcaμ33
GC1	114/158	251/251	298/310	307/322	245/264	160/218	174/198
GC2	116/130	255/255	310/310	329/331	251/256	211/213	174/174
Halg (Se3)	126/126	251/257	310/310	307/334	236/245	160/164	174/240
Hhip range (Melenara)	136–192	242–260	264–298	320–322	230–268	148–212	180–252
Hhip range (WS1 and Se1)	154–180	254–260	298	314–322	264	152–166	176–210

the same specimen was thus disproved. Microsatellites also confirmed that IDGC2 offspring was compatible with a full-sib group from a single breeding pair with the known father (GC2). The atypical genotyping associated to *H. algiricus* was proven for paternal alleles of offspring. By contrast, the maternal variants fit the patterns of allelic shape and range observed for *H. hippocampus* from Gran Canaria (López *et al.*, 2010). Beyond the concordant allelic range in West African specimens of *H. hippocampus* (Table II), they showed new alleles (*Hgu*-USC13*314 and *Hcaμ*33*176) which were not previously found in Canary populations of the species (López *et al.*, 2010), and they were also absent in the IDGC2 offspring. In spite of the restricted sample size that preclude population assignment of maternal origin, the nuclear DNA analysis brings additional support to the GC2 offspring as first generation hybrids between *H. algiricus* and *H. hippocampus* based on the maternally inherited *cytb* haplotype.

Morphometric and genetic analyses therefore confirm the first records of *H. algiricus* in the Canary Archipelago for two consecutive years (2008 and 2009), being the most northerly live specimens reported in the Atlantic Ocean coasts (Wirtz *et al.*, 2007). Moreover, these results have significant implications for the species' conservation as *H. algiricus* remains one of the species heavily involved in dried seahorse trade (Vincent *et al.*, 2011), and has been assessed as vulnerable in the IUCN Red List (Czembor, 2012).

Natural or anthropogenic causes could explain the presence of *H. algiricus* in Gran Canaria, as previously discussed for the lined seahorse *Hippocampus erectus* Perry 1810 in the Azores (Woodall *et al.*, 2009). Specimens of *H. algiricus* could have migrated passively with the Canary Current, West African coastal upwelling and the trade winds (Barton *et al.*, 1998). Alternatively, these records could also have an anthropogenic origin related to sporadic ship water ballast transport (Brito *et al.*, 2005). Release from private aquaria is unlikely because *H. algiricus* is not a common species in ornamental aquaculture (Koldewey & Martin-Smith, 2010). Regardless of the dispersal mechanism, climatic warming in the Canary region (Brito *et al.*, 2005; Hernández, 2006) noted over the last several decades could also favour the presence of *H. algiricus*.

The occurrence of *H. algiricus* beyond its previously documented geographic range may have consequences for local ecosystem diversity and structure (Gardner, 1997), especially in the light of the hybridization reported here. Despite the high genetic divergence found between *H. algiricus* and *H. hippocampus* (14·2% at *cytb*), within the interspecific range reported in seahorses (Casey *et al.*, 2004), an interbreeding event between both species was detected in this study. Furthermore, this finding represents the first hybridization event by members of the genus *Hippocampus* (seahorses) reported in the wild.

Interspecific mating could be explained by the seahorse mate recognition system, which is related to assortative size in breeding pairs (Jones *et al.*, 2003). The L_S of the two specimens of *H. algiricus* observed (129 and 134 mm) are close to those reported in adults of *H. hippocampus* living in the area (Otero-Ferrer, 2012, in press). GC2 could have sought a mating opportunity with a similarly sized individual (Yaakub *et al.*, 2006). On the other hand, hybrids can exhibit intermediate characteristics between those of their hybridizing parents, probably explaining the biometric and meristic values measured in the GC2 offspring (Kelly *et al.*, 2010). The existence of introgression was unevaluated given the sample size available in this study; however, genetic signs of hybrid adults were not found in populations of *H. hippocampus* in the same area (López *et al.*, 2010). Hybrid adults were neither detected during interbreeding episodes between the syngnathid species *Syngnathus auliscus* (Swain 1882) and *Syngnathus leptorhynchus* Girard 1854, and it has been associated with mechanisms of post-zygotic incompatibility and low hybrid viability, since interspecific hybrids were only detected at early embryonic stages (Wilson, 2006).

Research is needed to determine the extent of the hybridization events observed here, and to confirm whether the two specimens of *H. algiricus* observed in Gran Canaria are members of a previously unknown and established population, as opposed to occasional vagrants or recent invaders.

In summary, these results suggest an expansion respect to the known distribution range for *H. algiricus*, a species that is poorly understood but heavily traded, and the possibility of interbreeding events with sympatric populations of *H. hippocampus*. These findings should help to establish future regional conservation strategies that take into account this potential sympatric scenario between *H. algiricus* and *H. hippocampus*.

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