Article

A new species of seahorse (Teleostei: Syngnathidae) from the South China Sea

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Abstract

A new species of seahorse, *Hippocampus casscsio* **sp. nov.** was collected over shallow seagrass beds in Beibu Bay, China. This species is diagnosed from all other seahorse species by morphological characters, including the lower number of tail rings (35); 15 pectoral-fin rays; 16 dorsal-fin rays; a rounded nuchal plate without a raised coronet; a snout length 30% head length; two cheek spines and a dark brown coloration. In addition, molecular analysis showed all individuals of the new species clustering together suggesting a monophyletic lineage. This combined analysis supports the distinctness of *H. casscsio* **sp. nov.** as a new species, which is described herein.

Key words: taxonomy, *Hippocampus casscsio* **sp. nov.**, morphology, genetic distance

Introduction

Seahorses (genus *Hippocampus* Rafinesque 1810, family Syngnathidae) are a very distinct group of marine fishes. For centuries, people have been fascinated by their unique appearance and unusual reproductive strategy. They exhibit highly specialized phenotypes, such as a "horse-shaped" head, an elongated snout, absence of pelvic fins and caudal fin, and a prehensile tail, which distinguishes them from other teleostean fishes. They are also unique among vertebrates due to their 'male pregnancy' whereby males nourish developing embryos in a brood pouch until hatching and parturition occurs (Wilson *et al.*, 2001). About 41 valid seahorse species and over 400 pipefish species have been described the majority inhabiting shallow seabed of the Indo-central western Pacific Oceans (below latitude 26°N) (Dawson, 1985; Koldewey & Martin-Smith, 2010; Lourie *et al.*, 2005; Lourie *et al.*, 2016). Syngnathid taxonomy has been mainly based on morphological characters; however, a considerable degree of skill and taxonomic expertise is required to identify species, and often makes identification challenging (Gutiérrez *et al.*, 2014). More recently molecular methodology has been used to explore taxonomic issues and has been proved effective in revealing cryptic species (Bartlett & Davidson, 1991; Powers, 1991). A combination of morphological and genetic analyses offers the most powerful approach for seahorse taxonomy and phylogeny (Lourie *et al.*, 1999).

An undescribed species of *Hippocampus* was captured during recent fishery resources surveys along China's coast. The specimens are morphologically similar to *Hippocampus mohnikei* Bleeker 1853, a small, inshore species known from the Northeast Asian Seas (Lourie *et al.*, 1999; Masuda & Muzik, 1984). In Chinese waters it is found in the Bohai Sea and Yellow Sea. The new specimens were taken from Beibu Bay more than 2700 km southwest of the distribution of *H. mohnikei*. The new species described and named herein is compared to *H. mohnikei* and another similar species, *H. kuda* Bleeker 1852.

Methods and abbreviations

Most specimens were collected by researchers on board trawl boats, and a few were obtained with the help of local fishermen and buyers. Specimens cited in the present study are deposited in the Museum of the South China Sea Institute of Oceanology, Guangzhou, China (registration numbers SCSMBC007414-419).

We compared *H. casscsio* **sp. nov.** with the two most similar species, *H. kuda* and *H. mohnikei* using morphological characters. Morphometric measurements and counts were conducted following the standardized protocols of Lourie (2003) and Lourie & Randall (2003): SnL, snout length; HL, head length; TrL, trunk length; TaL, tail length; BH, body height; BW, body width; SL, standard length = HL+TrL+TaL; TrR, number of trunk rings; TaR, number of tail rings; PF, number of pectoral fin rays; AF, number of anal fin rays; DF, number of dorsal fin rays. Measurements were recorded to 0.1 mm and repeated to ensure accuracy. We measured 43 dried seahorses, and then all of the specimens were stored in alcohol. All the data was evaluated by one-way analysis of variance (ANOVA) followed by the Duncan's multiple-range tests. Difference were considered to be statistically significant if $P < 0.05$. Statistical analysis was carried out by using SPSS for Windows, Version17.0 (SPSS Inc., Chicago, IL, USA). The morphological data consisted of 11 metric and meristic characters from 43 adult specimens. A multivariate analysis of the morphological data set was implemented through a principal components analysis (PCA) in SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Phylogenetic relationships among *H. casscsio* **sp. nov.** and related seahorse species was determined by sequence analyses of the mitochondrial cytochrome *b* (cyt*b*) and control region (CR). DNA extraction, polymerase chain reaction (PCR) amplification, PCR sequencing, sequences assemblage and alignment followed the methods described in previous studies (Lourie & Vincent, 2004; Teske *et al.*, 2003; Zhang *et al*., 2014). Nucleotide sequences were assembled and edited using Bioedit 7.0.9.0 (Hall, 1999), and aligned using ClustalW (Larkin et al., 2007). Sequences were submitted to GenBank (accession numbers: cyt*b* for *H. kelloggi* Jordan & Snyder 1901 KT946908–KT946912, *H. kuda* KT946914–KT946915, *H. casscsio* **sp. nov.** KT946916–KT946918, *H. trimaculatus* Leach 1814 KT946919–KT946921, KT946930–KT946931, *H. spinosissimus* Weber 1913 KT946913, KT946922–KT946924, *H. comes* Cantor 1849 KT946925–KT946927, *H. barbouri* Jordan & Richardson 1908 KT946928, KT946933, *H. histrix* Kaup 1856 KT946934, KT946936 and *H. mohnikei* KT946929, KT946932, KT946935, KT946937; CR for *H. mohnikei* KT946938–KT946944, *H. casscsio* **sp. nov.** KT946945–KT946962 and *H. kuda* KT946963–KT946965). The numbers of haplotypes for each species were calculated using the software DnaSP 5.10.00 (Librado & Rozas, 2009). Sequence divergences were estimated following the Kimura two Parameter (K2P) distance (Kimura, 1980). The intraspecific distances and interspecific values within *Hippocampus* were calculated, respectively. An unrooted neighbor-joining (NJ) tree was created based on K2P distances using MEGA software (version 6.0) with bootstrap tests of 1000 replicates (Tamura *et al.*, 2013).

Comparative material. *Hippocampus kuda* Bleeker 1852. SCSMBC007416 (SL 90.5 mm, female), SCSMBC007417, 10 specimens (153.0 mm, female; 162.0 mm female; 160.0 mm, female; 171 mm, female; 167.5 mm, female; 183.0 mm, female; 180.0 mm, female; 168.5 mm, female; 179.0 mm, female; 176.0 mm, female), Dongshan, China (approximately 23°40' N,117°30' E), depth 10 m, collected by Qiang Lin, 23 Aug 2012.

Hippocampus mohnikei Bleeker 1853. SCSMBC007418 (SL 60.5 mm, female), SCSMBC007419, 15 specimens (62.0 mm, female; 74.0 mm male; 82.0 mm, male; 72.0 mm, male; 67.0 mm, male; 70.0 mm, male; 67.0 mm, female; 73.5 mm, male; 64.5 mm, female; 63.0 mm, male; 61.6 mm, female; 67.5 mm, male; 62.0 mm, female; 61.5 mm, female; 52.5 mm, female), Weihai, China (approximately 37°32' N, 122°13' E), depth 7 m, collected by Qiang Lin, 17 Aug 2013.

Systematics

Hippocampus casscsio sp. nov.
Beibu Bay seahorse
Figs 1, 2a, 3, 4, Table 1

Holotype. SCSMBC007414 (SL 90.5 mm, male), Beihai, Beibu Bay, China (approximately 21°20' N, 109°02' E), depth 15 m, collected by Qiang Lin, 12 May 2012. Paratypes: SCSMBC007415, 15 specimens (122.5 mm, female; 111.0 mm female; 132.0 mm, female; 104 mm, male; 100.5 mm, female; 106.5 mm, male; 88.0 mm, female; 126.5 mm, male; 129.0 mm, male; 133.0 mm, male; 121.5 mm, female; 117.0 mm, female; 102.5 mm, female; 118.0 mm, male; 120.5 mm, male), collection data as for holotype.

FIGURE 1. Holotype of *Hippocampus casscsio* **sp. nov.** (SCSMBC007414, SL 90.5 mm, male).

Diagnosis. *Hippocampus casscsio* **sp. nov.** is diagnosed from other seahorse species by the combination of the high number of tail rings (34–37), 15 pectoral-fin rays, 16 dorsal-fin rays (dorsal fins covering 2 trunk rings and 2 tail rings), a rounded nuchal plate without a raised coronet, the snout length approximately 30% in HL, one eye spine, two cheek spines, the tail length approximately 60% in SL, the plain dark brown body color, and male owning a fully enclosed brood pouch with a small opening for the incubation of eggs.

Description. In addition to the characteristics given in the diagnosis (holotype, paratype range in brackets): HL 15.8% (11.6–17.6%), TrL 28.2% (23.7–35.1%), TaL 56.0% (48.5–62.8%) in SL; SnL 30.5% (25.0–43.3%) in HL; TrR 10 (10–11), surface of TrR1, TrR4, TrR7 and TrR2 expanded laterally (but without spines); dorsal fin base starting immediately posterior to 9th trunk ring and ending immediately posterior to 2th tail ring (covering 2+2 rings); pectoral fin-base raised; DF 16; pectoral fin-base raised; PF 15; anal fin visible with 4 rays; first tail ring quadrangular; TaR 35 (34–37).

Body ornamentation: a prominent rounded spine above each eye, on midline of snout between eyes, and on either side of head below coronet; shoulder spine at base of pectoral fin; cheek spine.

Sexual dimorphism: males with closed brood pouch and slight keel anterior to dorsal fin base; greatly extended spines on inferior ridge on TaR 1–6th surrounding the brood pouch; females with slightly raised, circular genital opening; Males slightly larger SL, 116.0 mm (90.5–133.0 mm) compared to females 111.9 mm (88–132 mm).

Compared to other similar species, *Hippocampus casscsio* **sp. nov.** is notable for its short snout length and long tail length. Significant differences between *H. casscsio* **sp. nov.** and *H. kuda* specimens were detected in SnL/HL and TaL/SL ratios (Table 1). *H. kuda* also had a low-medium, rounded coronet with a cup-like depression on the top, which differed from the new species (Fig. 2a). *H. casscsio* **sp. nov.** is much larger than that of *H. mohnikei* (Fig. 2a).
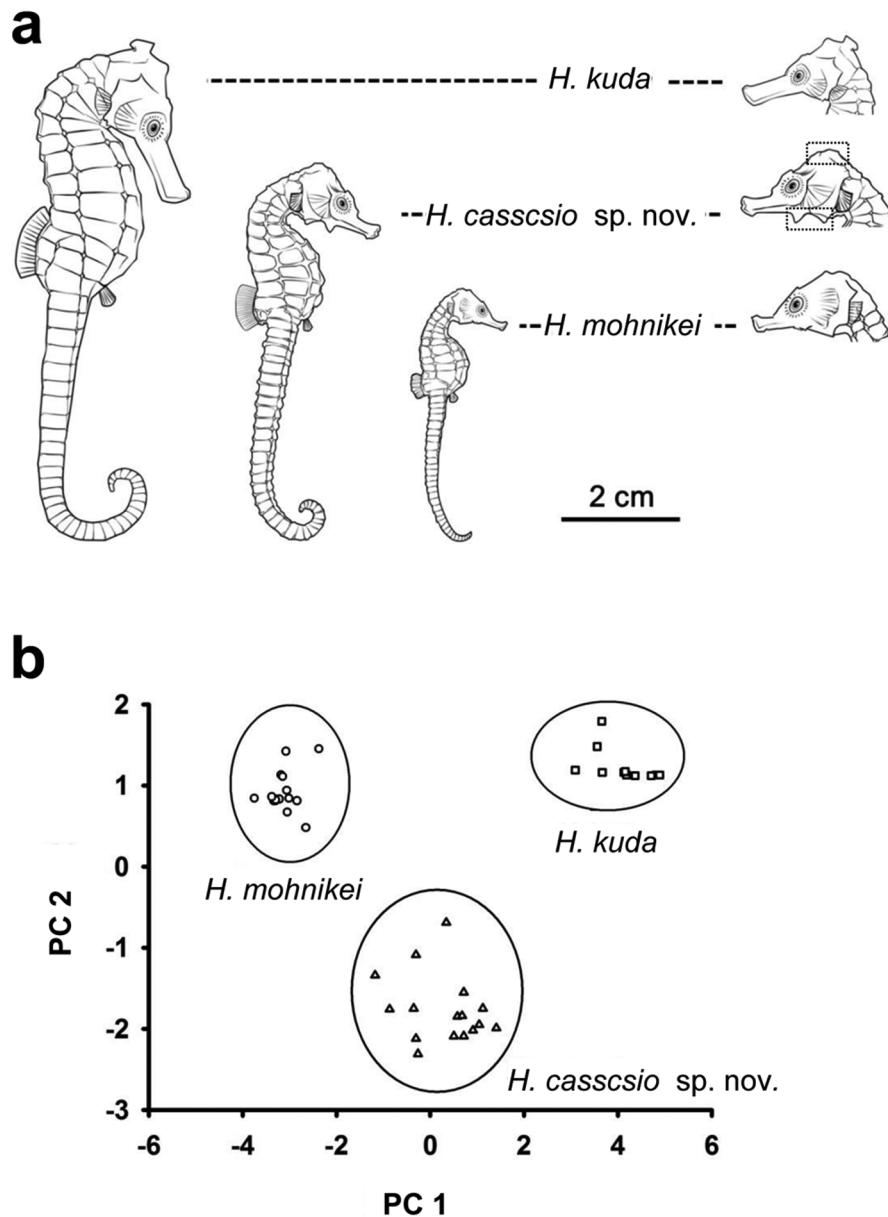

FIGURE 2. Comparison of morphological characteristics and body sizes among the three seahorses *H. kuda*, *H. casscsio* **sp. nov.**, and *H. mohinikei* (a), and the principal components analysis (PCA) of the three species according to the morphological data (b).

Principal component analysis showed that 11 metric and meristic characters integrated into 2 principal components and cumulatively contributed to 95.5% of total variance (Table 2). As the component matrix shows, the SL and BH had the highest factor loading in the first principal component, and the TrR had the highest factor loading in the second principal component. Standardized morphological data of all individuals were plugged into PCA function to calculate the score of the two factors. The score of the principal components is presented on Figure 2b. Ordination of the data using PCA showed no overlap among the three species seahorses included in this analysis.

TABLE 1. Morphological characteristics of three seahorses *H. casscsio* **sp. nov.**, *H. kuda* and *H. mohnikei*.

	Holotype*	*H. casscsio* **sp. nov.** (16)	*H. kuda* (11)	*H. mohnikei* (16)
Morphometrics				
SnL	4.5	5.4	15.3±0.64	2.4±0.53
HL	15.0	17.9±2.15	34.5±1.86	10.5±1.23
TrL	24.5	32.1±5.86	49.9±2.55	19.2±1.84
TaL	51.0	63.9±10.00	86.1±5.89	36.7±4.69
SL	90.5	113.9±14.07	170.5±9.97	66.4±7.11
BH	75.5	96.0±12.52	86.1±5.89	55.8±5.98
BW	14.0	17.1±2.92	21.2±2.52	8.7±1.24
SnL/HL	0.30	0.3±0.04[b]	0.4±0.01[a]	0.2±0.03[c]
TrL/SL	0.27	0.3±0.04[a]	0.3±0.01[a]	0.3±0.02[a]
	0.56	0.6±0.04[a]	0.5±0.01[b]	0.6±0.02[a]
HL/SL	0.17	0.2±0.01[a]	0.2±0.00[a]	0.2±0.01[a]
Meristics				
PF	15	15	16	13
DF	16	16	17	16
AF	4	4	4	4
TrR	10	10	11	11
TaR	35	35(33–37)	36(36–38)	38(37–40)

Sample size for each species was shown in parenthesis. Means (±S.E.) marked with different letters were significantly different from each other ($P < 0.05$). SnL, snout length; HL, head length; TrL, trunk length; TaL, tail length; BH, body height; BW, body width; SL, standard length. PF, number of pectoral fin rays; AF, number of anal fin rays; DF, number of dorsal fin rays; TrR, number of trunk rings; TaR, number of tail rings. * The data are for the holotype of *H. casscsio* **sp. nov.**

TABLE 2. Loadings of components matrix and contributions of each axis to total variance.

	Principal components	
	1	2
HL	0.974	0.200
SnL	0.950	0.289
TrL	0.976	0.068
TaL	0.973	-0.089
BH	0.994	-0.030
SL	0.997	0.022
BW	0.947	-0.210
PF	0.941	-0.214
DF	0.837	0.517
TrR	-0.075	0.961
TaR	-0.521	0.770
Variance (%)	77.174	18.290

SnL, snout length; HL, head length; TrL, trunk length; TaL, tail length; BH, body height; BW, body width; SL, standard length. PF, number of pectoral fin rays; DF, number of dorsal fin rays; TrR, number of trunk rings; TaR, number of tail rings.

Molecular analysis. All cyt*b* sequences were trimmed to a consensus length of 809 bp. A total of 30 unique cyt*b* haplotypes were generated from all specimens, and three haplotypes were observed among the new species. Eighteen CR haplotypes were observed among the specimens of *H. casscsio* **sp. nov.** The NJ tree showed that all of the individuals of the same species cluster together (Fig. 3a, b). No haplotype was shared between species in our data set. Although *H. casscsio* **sp. nov.** was morphologically similar to *H. mohnikei* (interspecific distance: 16.1%), there was a closer genetic relationship between *H. casscsio* **sp. nov.** and *H. kuda* (interspecific distance: 2.4%) (Fig. 3a and Table 3). The two species formed a sister group with a strongly significant bootstrap value of 100% (Fig. 3).

Etymology. The name is derived from the abbreviation of 'South China Sea Institute of Oceanology, Chinese Academy of Sciences'.

Distribution. *Hippocampus casscsio* **sp. nov.** appears to be relatively limited to the Beibu Bay (Beihai, Fangchenggang, Lingao, Dongfang, Sanya and Lingshui), China (Fig. 4).
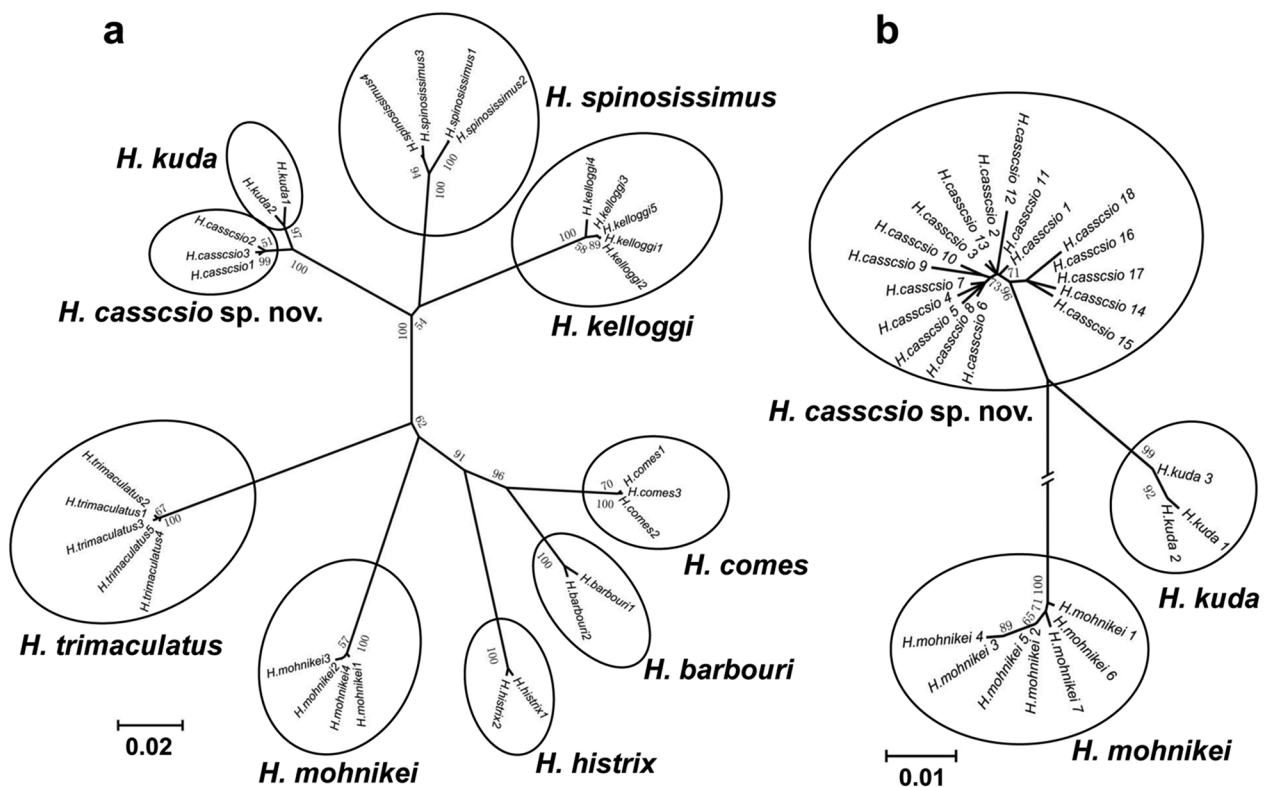

FIGURE 3. Neighbor-joining (NJ) trees based on cyt*b* (a) and CR (b) from seahorses haplotypes of *Hippocampus* along China's coast.

TABLE 3. Genetic distances based on cyt*b* among the nine seahorse species obtained along China's coast.

|	*H. kelloggi*	*H. comes*	*H. spinosissi*	*H. kuda*	*H. barbouri*	*H. histrix*	*H. trimaculat*	*H. mohnikei*	*H. casscsio* **sp. nov.**
H. kelloggi	0.004								
H. comes	0.172	0.001							
H. spinosissimus	0.110	0.159	0.013						
H. kuda	0.117	0.153	0.115	0.005					
H.barbouri	0.160	0.070	0.162	0.158	0.009				
H. histrix	0.186	0.111	0.167	0.177	0.118	0.005			
H. trimaculatus	0.185	0.166	0.159	0.172	0.166	0.160	0.002		
H. mohnikei	0.178	0.144	0.167	0.165	0.143	0.153	0.166	0.003	
H. casscsio **sp. nov.**	0.116	0.152	0.114	**0.024**	0.158	0.188	0.173	**0.161**	0.003
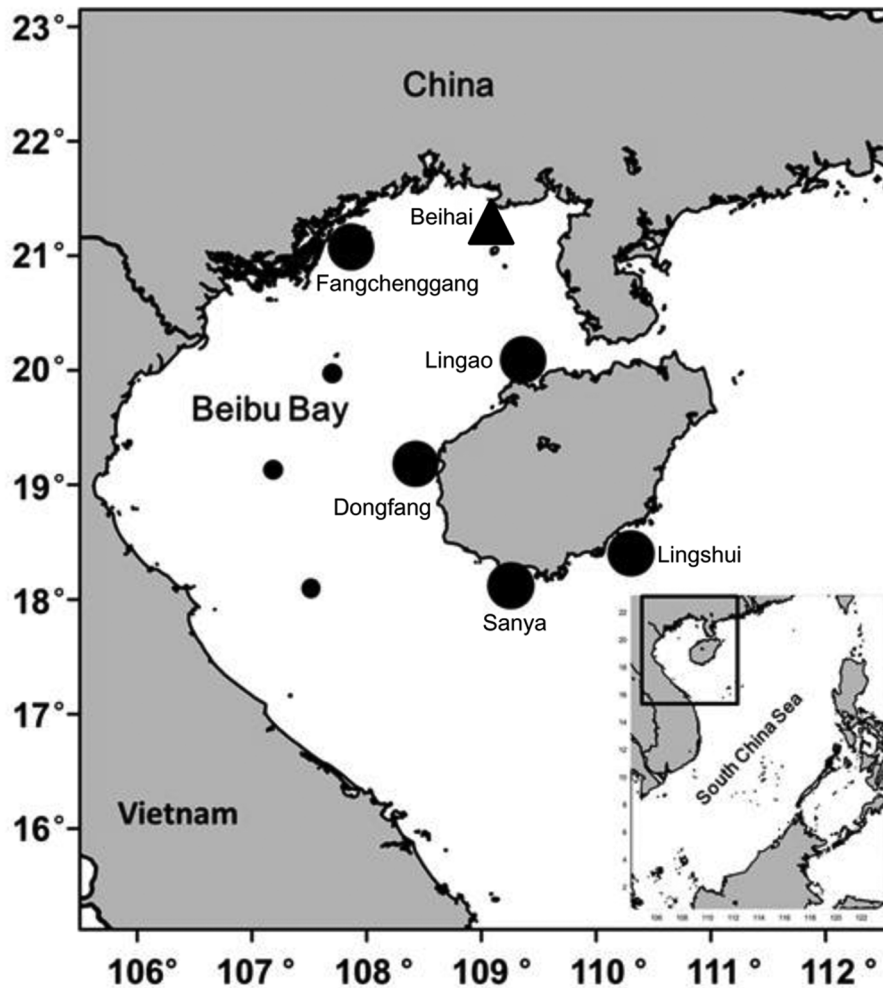
FIGURE 4. Map generated by Surfer® for Windows (Keckler, 1997) showing the sampling locations for the specimen of new seahorse species, *H. casscsio* **sp. nov.**, along China's coast. Holotype location indicated by triangle.

Discussion

The morphometric statistics addressed in this study indicated some differentiation among three seahorses *H. casscsio* **sp. nov.**, *H. kuda* and *H. mohnikei*. The new species has a relative low number of tail rings (mean = 35); however, based on 80 specimens, a previous study showed that the number of tail rings in *H. kuda* ranged from 34 to 38 (Lourie *et al*., 1999). This result suggests that tail ring number is a weak character to distinguish *H. casscsio* **sp. nov.** from *H. kuda*. The utility of morphological traits in diagnosis of species of *Hippocampus* can be problematic because some characteristics such as fin-ray number and trunk-ring number may vary greatly within any given-species (Horne, 2001; Lourie *et al*., 1999). However, morphological variation, such as the rounded coronet, was sufficient to distinguish *H. casscsio* **sp. nov.** from *H. kuda*, although the inter-group distances was small (2.4%). In the recent global revision of the seahorse taxonom, they adopted a 2% threshold for differences when assessing genetic information (Lourie *et al*., 2016).

Hippocampus casscsio **sp. nov.** and *H. mohnikei* specimens are also morphologically similar, resulting in potential misidentification. Compared with a maximum size of 88–133 mm SL in *H. casscsio* **sp. nov.**, Lourie et al. (1999) found that adult *H. mohnikei* ranged from 50 to 80 mm SL, implying that this characteristic is suitable to distinguish adult specimens. The genetic results also supports the distinctiveness of *H. casscsio* **sp. nov.** from *H. mohnikei*. In comparison with other seahorse species from China (*H. kelloggi, H. comes, H. spinosissimus, H. barbouri, H. histrix,* and *H. trimaculatus*), *H. casscsio* **sp. nov.** and *H. mohnikei* had higher genetic distance (interspecific distance: 16.1%), and unique mtDNA (cyt*b* and CR) haplotypes had become fixed.

In this study, dried seahorse specimens were utilized in the morphometric analyses. A previous study suggested that dried and alcohol-preserved seahorses can be used in identifying seahorses (Lourie *et al.*, 1999).

In conclusion, *H. casscsio* **sp. nov.** is genetically monophyletic, and morphometrically separated from *H. kuda* and *H. mohnikei*.

Acknowledgements

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