First Record and Morphological Identification of the Seahorse *Hippocampus kelloggi* Great Seahorse Jordan and Snyder (1902) in Coastal Waters of Pakistan

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Abstract

The first record of seahorse *Hippocampus kelloggi* commonly known as the great Seahorse from the coastal waters of Pakistan based on morphological and morphometric characteristics. The Seahorses belong to family Syngnathidae and about 50 species have been reported across the world. They have been found to inhabit coral reefs, seagrass beds and also coastal mangroves. All seahorse species (*Hippocampus* sp.) are vulnerable and according to the global conservation status of *Hippocampus kelloggi* is also among them. *H. kelloggi* is listed as data deficient since 2003 and then classified as IUCN Red List ([http://www.iucnredlist.org](http://www.iucnredlist.org)) Category & Criteria: A2cd version 3.1 (2017). The *Hippocampus kelloggi* previously recorded from the Indo Pacific regions, including China; India; Indonesia; Japan; Malaysia; Philippines; Thailand; United Republic of Tanzania; Viet Nam. The current study confirmed the presence of *H. kelloggi* from the coastal waters of Pakistan.

Keywords: Seahorse; Syngnathidae; *Hippocampus*; IUCN Red List; Pakistan

Introduction

This is the first record of seahorse *Hippocampus kelloggi* commonly known as the great seahorse from the coastal waters of Pakistan as provide morphological identification and range extension of the species. The seahorses are representative of family Syngnathidae that includes, pipe fishes, pipe horses and sea dragons. There are about 50 species reported across the world and have been found to inhabit various home ranges, i.e., Sea grass beds, coastal mangroves, and coral reefs, although characterized as sedentary fish species with patchy distribution [1-3]. *H. kelloggi* previously described for the low mobility, fecundity, prolonged parental care, mate fidelity and reproduction information [4-6]. In Philippines, *H. kelloggi* reported at maximum depth of 152 m and associated morphological revisions of seahorse have been also carried out [7]. According to the morphometric characteristics, five species of *Hippocampus* including *Hippocampus kuda* (Bleeker 1852), *H. fuscus* (Ruppell 1838), *H. trimaculatus* (Leach 1814), *H. kelloggi*, and *H. histrix* (Kaup 1856) reside in the Indian Ocean waters [8].

All *Hippocampus* species are included in the IUCN Red List ([http://www.iucnredlist.org](http://www.iucnredlist.org)). According to the IUCN Red List (2006) 20 species of seahorse designated as ‘vulnerable’; 11 as ‘data deficient’ and one (*H. capensis*) designated as endangered. *H. kelloggi* is listed as data deficient since 2002 by IUCN and vulnerable (VU) as assessment done in 2017 by IUCN [9]. The assessment designated *H. kelloggi* as vulnerable species in Red List Category and Criteria: A2cd ver. 3.1 in the IUCN Red List of threatened species and included in the CITES Appendix II effective in May 2004, although the *H. kelloggi* removed from the Australian Wildlife Protection Act in 1998 and then placed in Environment Protection and Biodiversity Conservation Act in 2001. However, according to the review of China’s biodiversity reported *H. kelloggi* as the threatened species of China as placed in wildlife protection.
laws, while the Priority Fish species (Grade B) also included *H. kelloggi* in National Red Data book, as a vulnerable in the Viet Nam [5].

Dried seahorse used in the traditional Chinese medicine market. The trade of seahorse observed throughout its distribution range bycatch during shrimp trawling [10-13]. Whereas currently legal and illegal trade also enduring (UNEP-WCMC 2012 a, b) that why the stock was substantially declines between 1995 and 2000 [6], in addition Singapore, United Arab Emirates and Hong Kong import 4.34 tons of sea horse from India during the year 2001–200 [5,14-16].

According to 70% decline in Seahorse population included *H. kelloggi* [12,15,17] due to the over exploitation at the Palk Bay and Gulf of Mannar in the South-east coast of India, and to overcome this issue, India Government prohibited the trade and fishing of representatives of family Syngnathidae as a protected species under the Schedule I (Part 2A) of the Indian Wildlife (Protection) Act, 1972 through a Notification No. 1-4/95 WL1 dated 11 July, 2001, [12,15], UNEP-WCMC 2012b. Molecular identification of dried seahorse and pipe fish was done, that was sold on the Chinese market by using two mitochondrial *COI* and *16S rRNA* genes and revealed the exploitation of representatives of family Syngnathidae by illegal and not regulated trade. Previously, there is no record of sea horse species from the coastal water of Pakistan, the current study provides the evidence for the presence and range extension of *H. kelloggi* from the coastal water of Pakistan [18,19].

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**Hippocampus kelloggi** Jordan and Snyder 1902

**Common names**

Kellogg’s Seahorse, Great Seahorse (U.S.A.), Offshore Seahorse (Viet Nam), O-umi-uma (Japanese; Japan)

**Synonyms**

*H. suezensis* Duncker (1940)

**Systematics**

Actinopterygii (ray-finned fishes)

Syngnathiformes (Pipefishes and seahorses)

Syngnathidae (Pipefishes and seahorses)

Hippocampinae

**Etymology**

*Hippocampus*: Greek, Ippos = horse; Greek, kampe = curvature.

**Colour/pattern**

Greenish brown in color.

**Confirmed distribution**

According to [18] distributed throughout the Indo Pacific region, including China; India; Indonesia; Japan; Malaysia; Pakistan; Philippines; Thailand; United Republic of Tanzania; Viet Nam (Figure 1).

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**Figure 1**: Distribution range of *H. kelloggi* Ref: I Naturalist September 2019.
Habitat

Maximum reported depth 152 m; associated with gorgonian corals and sea whips; soft bottom Microalgae on rocky reefs. Seashores are found in temperature and tropical coastal waters, coral reefs, and seagrass beds.

Economic Importance

Dried for traditional medicine and curios, live for aquarium and hobbyist use.

Vulnerability

Red List Category & Criteria: Vulnerable A2cd ver 3.1 (2017)
Red List Vulnerable (VU) (2012)
http://dx.doi.org/10.2305/IUCN.UK.2012.2.LTS.T41010A17242053.en Data Deficient (DD) (2002). (Table 1).

Table 1: Conservation status.

| Conservation Status | CITES | IUCN | Appendix II effective May 2004 |
|---------------------|-------|------|-------------------------------|
| Australian Wildlife Protection Act |   |      | Removed in 1998 |
| Environment Protection and Biodiversity Conservation Act |   |      | Placed in 2001 |
| Wildlife Protection Laws |   |      | Priority Fish species (Grade B) Review of China's biodiversity |
| National Red Data Book |   |      | Vulnerable in the Viet Nam |

Morphological Identification and Morphometric Measurements

The description of the seahorse was made based on the observation of a single specimen which was caught in a rock pool at Mubarak village, rocky cum sandy shore found in the rocky pool at a depth of four feet, from the coastal waters of Pakistan (Figure 2), on dated 18th April 2019. Morphological identification based on the taxonomic characters and all Morpho-measurements were made according to the standard protocol. Length of specimen is given as standard length (from the tip of snout to end of the tail) up to the nearest 0.1 mm. The comparison of morphometric and meristic character was made based on the earlier literature [7]. Later the Morphometric characters were examined and compared with earlier observations (Table 2). As per earlier description, this species shares the characters similar to the Hippocampus spinosissimus with the presence of distinct high coronet and with five short spines.

Table 2: Morphological Measurements of H. kelloggi.

| Morphological variables | Measurements |
|-------------------------|--------------|
| 1 Cheek spines (CS)    | Prominent slightly backward Pointing cheek spines |
| 2 Cleithral ring       | yes          |
| 3 Coronet              | high with five short spines |
| 4 Dorsal fin rays      | 18           |
| 5 Eye spines (ES)      | Prominent Eye Spines |
| 6 Head length (HL)     | 2 cm         |
| 7 Height (H)           | 9.9 cm       |
| 8 Nose spine           | yes          |
| 9 Operculum            | yes          |
| 10 Pectoral fin rays   | 17           |
| 11 Snout length (SnL)  | 0.8 cm       |
| 12 Tail length         | 6.4 cm       |
| 13 Tail rings (TaR)    | 39 rings     |
| 14 Trunk length        | 2.6 cm       |
| 15 Trunk rings (TrR)   | 11           |
| 16 Rings supporting dorsal fin | 2 Trunk rings, 1 Tail ring |
| 17 Anal fin            | Yes          |
Diagnostic characteristics (Figure 3 & 4).

Seahorse species have elongated body encased in thick trunk rings, back-pointing, small pectoral fins and a single dorsal fin, thick snout with no teeth, maximum recorded height is 28 cm, with tiny white spots running in vertical lines. Body narrows with thick snout (tube like), deep head. The species characterized by the distinct high coronet, with five short spines with high plate in front of coronet and a prominent rounded eye spine. They have long, slightly backward pointing, rounded cheek spine. Spines low and rounded; slightly better developed in younger specimens, but still blunt tipped. Other distinctive characteristics: Long, slightly backwards-pointing, rounded cheek spine; deep head; narrow body; thick body rings; prominent, rounded eye spine; thick snout.

Figure 2: Map of the occurrence (Mubarak village) of H. kelloggi from the coastal waters of Pakistan.

Figure 3: Lateral view of complete specimen of H. kelloggi.
Taxonomic Remarks

According to literature [5], Pakistan included in the distribution range of H. kelloggi however, there is no previous record of H. kelloggi from the coastal waters of Pakistan, the current study is the first detailed study and describe the range extension of H. kelloggi.

Molecular Study

SDS and Native PAGE (Polyacrylamide gel electrophoresis) of General protein (GP) EC (non-specific)

Seahorses are economical, commercially important and traded internationally, used in traditional Chinese medicine and its derivatives for the treatment includes: the respiratory disorders (asthma), sexual dysfunctions (Erectile dysfunction), and general lethargy due to the insufficient sleep, overexertion, overburdening, stress, lack of exercise, improper nutrition, boredom, and pains [10]. During the present study, an estimation was made for the various size of proteins by using the electrophoresis technique. Two types of electrophoresis were performed, SDS for general protein and Native PAGE for the specific protein (specific protein by using the specific subtrate of enzyme). SDS and Native PAGE (Polyacrylamide gel electrophoresis) of General protein (GP) EC (non-specific) banding pattern was estimated in muscle tissue of K. kelloggi. Approximately 250-300 mg was removed and homogenized in 1 ml of Tris Citrate II extraction buffer, and the sample was centrifuged at 15,000 rpm for 15 minutes, supernatant was used as protein source.

Electrophoresis was performed [20] accordingly, whereas the gel was stained (Coomassie Brilliant Blue) for General protein (GP) EC (non-specific), Bovine (BSA serum albumen) used as standard protein. Electrophoresis was performed in vertical native polyacrylamide gels (Native-PAGE) as described by [20]. A total of four protein bands were observed in SDS PAGE electrophoresis (Figure 5), the Rf values ranges from 0.08 to 0.451, the standard band (5 KDa standard of BSA) was observed in between gel that showed that the higher and lower molecular weight protein was observed in H. kelloggi sample whereas a total of five protein bands were observed in Native PAGE electrophoresis (Figure 5), the Rf values ranges from 0.098 to 0.633. The further detailed biochemical study and characterization of protein can reveal some interesting results from these species.
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Figure 5: SDS (A) and Native PAGE (B) (Polyacrylamide gel electrophoresis) of General protein (GP) EC (non-specific) banding pattern of H. kelloggi.

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