A new seahorse species of *Pegasus* (Syngnathiformes: Pegasidae) from the East China Sea

**DEAR EDITOR,**

The Pegasidae family, which includes seven species of seahorses, is widely distributed in the temperate and tropical waters of the Indo-Pacific, ranging from South Africa to Hawaii. In this study, we describe a new species of seahorse, *Pegasus sinensis* sp. nov., based on morphological and molecular characterization of 14 specimens collected from the surrounding waters of Xiamen City, Fujian Province, China. The new species differs from closely related *P. volitans* in both the density and color of spots present on the dorsal and lateral body surfaces. Based on the mitochondrial genomes (mitogenomes) of three newly sequenced pegasids used in this study, we conducted a phylogenetic analysis of Pegasidae, revealing that *Pegasus sinensis* sp. nov. and *P. volitans* diverged from their common ancestor approximately 9.0 million years ago (Ma). Two members of the pegasus genus *Spinipegasus* (*S. laternarius* and *S. nanhaiensis*) clustered outside the monophyletic branch of *Pegasus* and *Eurypegasus*, supporting the classification of *Spinipegasus* as a valid genus, as proposed in previous studies.

The order Syngnathiformes consists of 10 families, including the large and diverse family Syngnathidae, which contains 320 species of seahorses, seadragons, and pipefish (Stiller et al., 2022). In contrast, only seven species are currently recognized in the family Pegasidae, including two new species (*P. tetrabelos* and *S. nanhaiensis*) described in 2016 and 2020 (Osterhage et al., 2016; Zhang et al., 2020). Millions of pegasids are sold each year as traditional Chinese medicine (Pajaro et al., 2004), and further research is needed to estimate their species diversity and population abundance across their distribution range.

Seahorses are widely distributed in the temperate and tropical waters of the Indo-Pacific, ranging from South Africa to Hawaii (Pajaro et al., 2004; Zhang et al., 2020). The family Pegasidae contains seven species in two genera: i.e., *E. draconis* and *E. papilio* in *Eurypegasus* (restricted to Hawaii, USA) and *P. volitans* Linnaeus, 1758, *P. laniger* Kaup, 1861 (restricted to southern Australia), *P. laternarius* Cuvier, 1829, *P. tetrabelos* (northern Australia; Osterhage et al., 2016), and *P. nanhaiensis* (southern China; Zhang et al., 2020) in *Pegasus*. The subgenus *Spinipegasus* was erected by Lindberg et al. (1997) but was later suggested to be elevated to the rank of genus (Prokofiev, 2017). At present, however, the species *S. laternarius* is currently recognized as *P. laternarius* in most fish databases and literature (Osterhage et al., 2016; Pajaro et al., 2004).

*Pegasus volitans* was originally named and described by Linnaeus, 1758. Three names have been proposed for this species, i.e., *P. draconis*, *P. volans*, and *P. natans*; however, upon further investigation, none of these classifications could be defined as a valid species in the genus *Pegasus* (Osterhage et al., 2016; Zhang et al., 2020). The closely related congener *P. draconis* was later transferred to the genus *Eurypegasus* (Osterhage et al., 2016). According to detailed description of the species, *P. volans* and *P. natans* are now considered synonyms of *P. volitans* (Osterhage et al., 2016; Zhang et al., 2020).

From 2020 to 2021, we collected hundreds of pegasids from the coastal regions of South China by bottom trawling, including 14 *Pegasus* specimens from the waters surrounding Xiamen City, Fujian Province, China. Abdominal muscle tissue was extracted and stored at −80 °C for molecular analysis, and fresh fish were stored in absolute ethanol after fixation in a 10% formalin solution (Osterhage et al., 2016). All specimens were freeze-dried for long-term preservation and deposited in the South China Sea Marine Biological Museum of the Chinese Academy of Sciences.

Received: 18 May 2022; Accepted: 05 July 2022; Online: 06 July 2022

Foundation items: This work was supported by the Key Special Project for Introduced Talents Team of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (GML2019ZD0401, GML2019ZD0407), Guangdong Basic and Applied Basic Research Foundation (2019A1515101199), National Natural Science Foundation of China (42006109, 41825013), and Key Research Program of Frontier Sciences of CAS (ZDBS-LY-DOC004)
The 14 Pegasus specimens collected in Fujian differed from all known congeneres, including their sister species P. volitans. All 14 specimens were counted and measured following Osterhage et al. (2016) and Zhang et al. (2020). All measurements were made with a digital vernier caliper to the nearest 0.01 mm. Genomic DNA was extracted from abdominal muscle tissue using a TIANamp Marine Animal DNA Kit (Tiangen, China) according to the manufacturer’s instructions. DNA sequencing was performed using the Illumina HisEq 2500 system (Illumina, USA), and clean reads were assembled using MitoZ and metaSPAdes. The assembled mitogenome sequence was annotated using MITOS (http://mitos2.bioinf.uni-leipzig.de/index.py). Mitochondrial 16S rDNA gene sequences were obtained and amplified by polymerase chain reaction (PCR). Primers were designed using Primer Premier v5.0: 16S F (5’–3’) CCGCTGTTTATCAAAAACAT and 16S R (5’–3’) CCGGTCTGAACTCAGATCACGT. The 16S DNA gene fragments and all 13 mitochondrial protein-coding genes were aligned using MAFFT v7. The best nucleotide substitution model was selected using ModelFinder. Phylogenetic trees were constructed based on 16S rDNA and 13 mitochondrial genes using Bayesian methods in MrBayes v3.2.6. Molecular dating based on the 13 mitochondrial genes was performed using BEAST v1.4.6.

Taxonomic account

Pegasus sinensis sp. nov. (Figure 1B–D; Supplementary Table S1)

Holotype: Pegasus sinensis sp. nov., TMBC030944, 73.4 mm SL, collected from the coastal waters of Xiamen City, Fujian Province, China, August to October 2020 and April to June 2021.

Paratypes: Pegasus sinensis sp. nov., TMBC030945–030957, 65.02–75.59 mm SL, collected from coastal waters of Xiamen City, Fujian Province, China, August to October 2020 and April to June 2021.

Etymology: The species name sinensis is derived from its...
currently known distribution in the coastal waters of China.

Suggested English name: Chinese seamoth

Suggested Chinese common name: 中华海蛾鱼 (Chinese phonetic alphabet: zhōng huá hǎi é yú).

Diagnosis: Pegasus sinensis sp. nov. can be distinguished from all known congeners based on a combination of the following characters (see Supplementary Table S2 for details, with P. volitans, P. tetrabelos, S. laternarius, and S. nanhaiensis data taken from Osterhage et al., 2016; Zhang et al., 2020): (1) tail rings 12 (I–XII) (vs. 11 in S. laternarius and S. nanhaiensis); (2) body slender, similar to P. volitans, carapace width 14.44%–18.15% of SL (vs. 28.8%–37.0% of SL in S. nanhaiensis and 24.7%–32.2% of SL in S. laternarius); (3) rostrum length 21.01%–25.53% of SL (vs. 4.8%–17.4% of SL in S. nanhaiensis and 7.5%–19.9% of SL in S. laternarius); (4) pectoral fin rays 11 (vs. 10 in P. tetrabelos); (5) tail length 60.44%–65.76% of SL (vs. 59.3%–65.2% of SL in P. tetrabelos and 45.1%–52.8% of SL in S. nanhaiensis); (6) overlaid large, dark spots (vs. smaller brown to dark brown spots in P. volitans and P. tetrabelos); and (7) dorsal plate pairs 3 (dl₁–₃), dorsolateral plate pairs 4 (dl₁–₄), ventrolateral plate pairs 5 (vl₁–₅), caudodorsal plate pairs at tail 11, and caudoventral plate pairs 11 (Figure 1B–D).

Descriptions: Body flat and depressed, body depth of holotype 10.49% (9.23%–11.22% in paratypes) of SL, body depth generally constant. Carapace width 17.17% (14.44%–18.15%) of SL, prepectoral width 22.07% (20.43%–24.74%) of SL, interpectoral width 18.53% (15.56%–21.43%) of SL. Body width gradually tapering from mid-trunk to tail and from mid-trunk to triangular-shaped head. Head width 14.17% (13.67%–17.61%) of SL. Head width gradually tapering from anterior orbit to anterior suboral chamber. Rostrum long, rod-shaped, length 22.75% (21.01%–25.53%) of SL. Rostrum width relatively constant, increasing to 3% (2.65%–3.75%) of SL at rostrum tip. Interorbital width 5.86% (5.67%–6.92%) of SL, carapace length 37.47% (34.24%–39.56%) of SL, pairs of dorsal ridges extending from posterior of orbital and bisecting dorsal plate, gradually widening near caudal ring, extending into dorsolateral margin of tail. Carapace scaleless, with three pairs of dorsal plates (dl₁–₃) and four pairs of dorsolateral plates (dl₁–₄) connected to ventrolateral plates. Abdomen with five pairs of ventrolateral plates (vl₁–₅). Eleven pairs of caudodorsal plates at tail, connected to 11 pairs of caudoventral plates. Tail length 62.53% (60.44%–65.76%) of SL. Tail ring tapered and elongated to form flat posterior section (with 12 tail rings I–XII). Pectoral fins wing-shaped with 11 rays, fifth longest. Pelvic fin with one spine and two rays. Dorsal and anal fins with five rays, first longest and fifth shortest. Caudal fin with eight rays.

Color in life: Dorsal and lateral body surfaces light brown to beige, covered with dark brown to black spots. Approximately 20 spots on dl₁ and dl₂, approximately 30 spots on dl₂ with some smaller scattered spots. Plate connecting trunk to tail light brown, gradually lightening towards transparent tail ring XII. Tail covered with dark brown to black spots. Ventrolateral spots light brown to brown, slightly lighter than those on trunk. Color gradually darkening from trunk to head. Head bright cream, gradually lightening towards off-white rostrum. Pupils black with dark gray orbits. Rostrum translucent with translucent light brown spots. Entire ventral surface off-white to white with spotless covering, some dorsal saddles visible ventrally through tail rings, especially posteriorly. Pectoral fins translucent and pectoral fin bases light brown. Dark brown spots, variable in size, present on pectoral fins and pectoral fin rays. Large spots distributed in rows in middle of pectoral fin and near outer membrane of fin, with other spots similar in size to those on trunk. Pelvic and anal fins translucent without obvious spots. Dorsal and caudal fins translucent with dark brown spots on rays (Figure 1B–D).

Color in preservative: Dorsal and lateral body surfaces pale yellow with numerous dark brown to black spots. Trunk (especially near head) dark gray. Head dark tan to dark gray. Ventral body surface light yellow.

Distribution: The new species is currently only found in Xiamen Bay, Fujian Province, China (Figure 1A).

Morphological comparison: Pegasus sinensis sp. nov. and P. volitans can be distinguished based on the following differences in spots present on dorsal and lateral body surfaces: (1) density of spots lower in Pegasus sinensis sp. nov. than in P. volitans; and (2) spots dark in Pegasus sinensis sp. nov. vs. brown to dark brown in P. volitans. In addition, the new species is genetically divergent and forms an independent clade from its sister species and all other congeners based on mitogenome and 16S rDNA sequences.

Phylogenetic and genetic comparisons: The complete mitogenomes of Pegasus sinensis sp. nov. (16 512 bp length; GenBank accession No. OM960961), S. laternarius (16 429 bp; OM960960), and S. nanhaiensis (16 415 bp; OM960959) were newly sequenced in this study. All contained 13 protein-coding genes, two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and a control region (CR), and shared the same gene order. The phylogenetic relationships of the five Pegasidae species were determined using 13 mitochondrial gene sequences, with syngnathids as the outgroup. The best-model (GTR+F+I+G4) of the mitogenome sequence was determined by ModelFinder, and 20 000 000 MCMC generations was used in Bayesian analysis with a final average standard deviation of split frequencies below 0.01. Three calibration time points, Syngnathoides bicuculatus-Hippocampus reidi (~42.5–48.7 Ma), Hippocampus ingens-Hippocampus reidi (~3.1–4.6 Ma), and Pegasus volitans-Eurypegasus draconis (~5.7–27.5 Ma), were used as constraints in the MCMCTree estimation (Wang et al., 2019). The mitogenome phylogeny recovered P. volitans and Pegasus sinensis sp. nov. as sister species within Pegasus (Figure 1E), both of which diverged from their common ancestor ~9.0 Ma. Furthermore, S. laternarius and S. nanhaiensis diverged 3.9 Ma and were recovered outside the monophyletic branch of Pegasus and Eurypegasus. Both S. laternarius and S. nanhaiensis were previously considered to belong to the genus Pegasus (Osterhage et al., 2016; Zhang et al., 2020). However, our phylogenetic results suggested that Eurypegasus is the closest living relative of Pegasus, supporting the classification of Spinipegasus as a valid genus, as proposed by Prokofiev (2017).

Molecular phylogenetic analysis based on 16S rDNA (GenBank accession Nos.: Pegasus volitans, Pegasus sinensis sp. nov., and P. tetrabelos)
S.S.L., and X.W. performed the experiments and analyzed the data. X.W. and Y.Y.Z. prepared the manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

We are grateful to Dr. Zhi-Yun Chen for help with morphometrics.

Ying-Yi Zhang1,4, Rong-Rong Zhang3, Shao-Bo Ma1,4, Shuai-Shuai Liu1,2, Qiang Lin1,2,4,5,*, Xin Wang1,2,5,*.  
1 CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, Guangdong 510301, China  
2 Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, Guangdong 511458, China  
3 State Key Laboratory of Marine Environmental Science/College of the Environment and Ecology, Xiamen University, Xiamen, Fujian 361102, China  
4 University of the Chinese Academy of Sciences, Beijing 100049, China  
5 Laboratory for Marine Fisheries Science and Food Production Processes, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao, Shandong 266237, China  
*Corresponding authors, E-mail: linqiang@scsio.ac.cn; wangxin2014@scsio.ac.cn

REFERENCES

Lindberg GU, Fedorov VV, Krisayukova ZV. 1997. Fishes of the Sea of Japan and the Adjacent Parts of the Sea of Okhotsk and Yellow Sea, Part 7: Dactylopteriformes–Pegasiformes, Handbook on the Identification of Animals. Saint Petersburg: Zoological Institute of the Russian Academy. (in Russian)

Osterhage D, Pogonoski JJ, Appleyard SA, White WT. 2016. Integrated taxonomy reveals hidden diversity in northern Australian fishes: a new species of seamoth (Genus Pegasus). PLoS One, 11(3): e0149415.

Pajaro MG, Meeuwig JJ, Giles BG, Vincent ACJ. 2004. Biology, fishery and trade of sea moths (Pisces: Pegasidae) in the central Philippines. Oryx, 38(4): 432–438.

Prokofiev AM. 2017. New findings of rare fish species in Indian and Pacific oceans with the description of two new species from the families Gobidiidae and Phylmateidae. Journal of Ichthyology, 57(6): 803–820.

Stiller J, Short G, Hamilton H, Saarman N, Longo S, Wainwright P, et al. 2022. Phylogenomic analysis of Syngnathidae reveals novel relationships, origins of endemic diversity and variable diversification rates. BMC Biology, 20(1): 75.

Wang X, Zhang YH, Zhang HK, Qin G, Lin Q. 2019. Complete mitochondrial genomes of eight seahorses and pipefishes (Syngnathiformes: Syngnathidae): insight into the adaptive radiation of syngnathid fishes. BMC Evolutionary Biology, 19(1): 119.

Zhang RR, Wang X, Wan SM, Ma SB, Lin Q. 2020. A new species of Pegasus (Syngnathiformes: Pegasidae) from the South China Sea. Zootaxa, 4894(4): 521–534.