

Letter to the editor

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

DEAR EDITOR,

The Pegasidae family, which includes seven species of seamoths, 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 seamoth, *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 pegasid 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.

Seamoths 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. lancifer* 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.

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The 14 *Pegasus* specimens collected in Fujian differed from all known congeners, 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') CGCCTGTTTATCAAAAACAT and 16S R (5'–3') CCGG TCTGAACTCAGATCACGT. The 16S rDNA 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

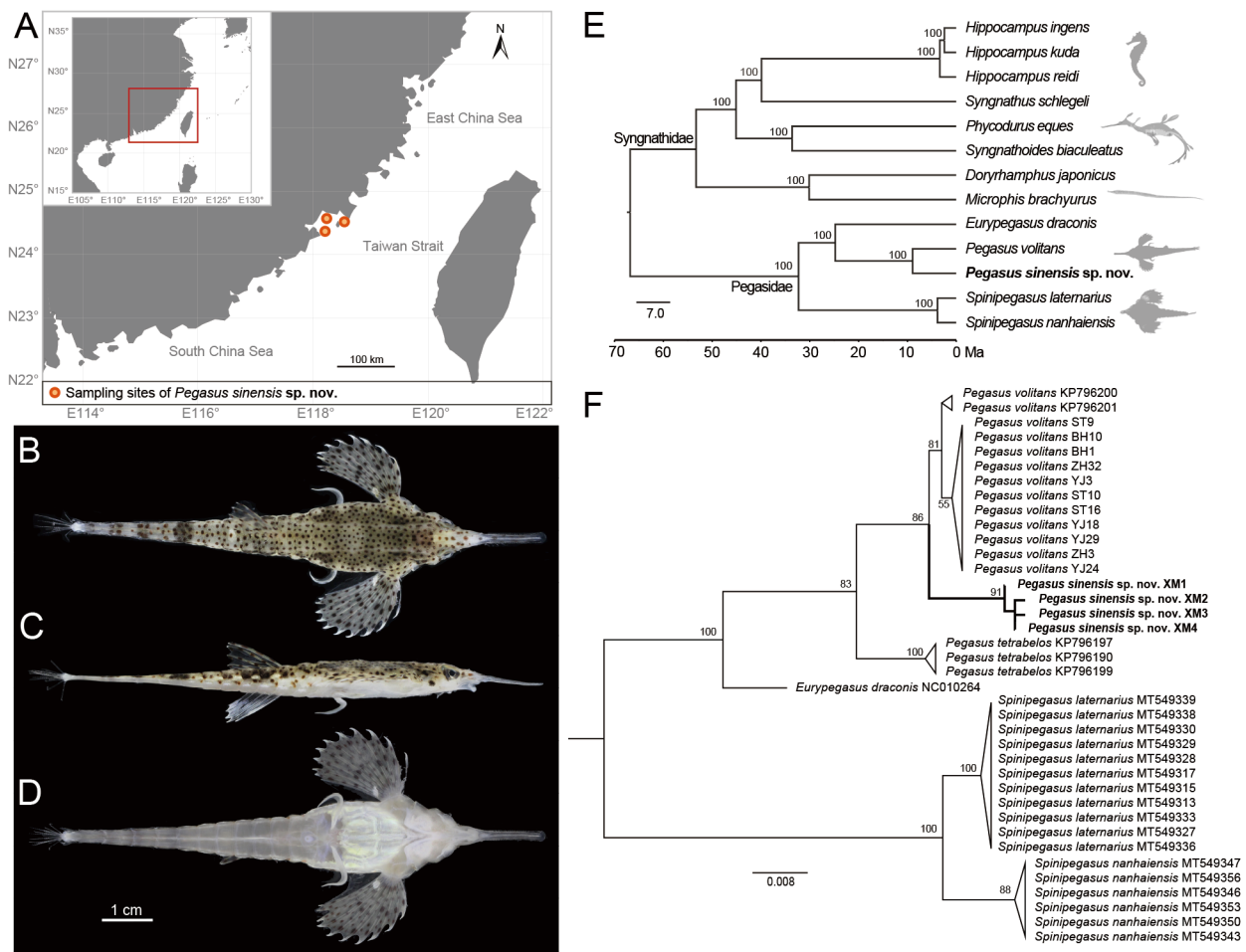


Figure 1 Sampling sites, holotype, and phylogeny of *Pegasus sinensis* sp. nov.

A: Sampling sites of *Pegasus sinensis* sp. nov. (red circles). B–D: Dorsal, lateral, and ventral views of *Pegasus sinensis* sp. nov. (TMBC030944). Photos by Xin Wang. E: Bayesian phylogram of Pegasidae inferred from mitogenome sequences. F: Bayesian phylogram of Pegasidae inferred from mitochondrial 16S rDNA.

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 (d_{1-3}), dorsolateral plate pairs 4 (dl_{1-4}), ventrolateral plate pairs 5 (vl_{1-5}), 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 subrostral 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 (d_{1-3}) and four pairs of dorsolateral plates (dl_{1-4}) connected to ventrolateral plates. Abdomen with five pairs of ventrolateral plates (vl_{1-5}). 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 d_1 and d_3 , approximately 30 spots on d_2 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-fit 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 biaculeatus-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*,

OM943755–OM943765; *Pegasus sinensis* sp. nov., OM957548–OM957551) (Figure 1F) revealed that *Pegasus sinensis* sp. nov. formed a strongly supported monophyletic group. The best-fit SYM+G4 substitution model was used for Bayesian analysis. Results showed that *Pegasus tetrabelos*, recently described by Osterhage et al. (2016), was clustered with *Pegasus sinensis* sp. nov. and *P. volitans*. Thus, *Pegasus sinensis* sp. nov. can be clearly distinguished from other members of *Pegasus* by both morphological and genetic traits. The *Pegasus* and *Spinipegus* genera were not recovered as a monophyletic clade in the molecular phylogeny, as supported by their morphological differences (see Diagnosis). Therefore, we consider *Spinipegus* as a valid genus with at least two species, i.e., *S. laternarius* and *S. nanhaiensis*.

NOMENCLATURAL ACTS REGISTRATION

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>.

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SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

All sampling and procedures involving seamounts were performed in accordance with the Wild Animals Protection Law of the People's Republic of China and approved by the Animal Ethics Committee of the Chinese Academy of Sciences (approval No. SCSIO-IACUC-2019-000137).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.W. and Q.L. conceived and designed the study. R.R.Z. and X.W. collected specimens in the field. Y.Y.Z., R.R.Z., S.B.M., 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.

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