

## Description and DNA Barcoding of a New *Sillago* Species, *Sillago shaoi* (Perciformes: Sillaginidae), in the Taiwan Strait

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**Jia-Guang Xiao, Na Song, Zhi-Qiang Han, and Tian-Xiang Gao (2016)** Reliance only on morphology to identify fishes to the species level is challenging when the diagnostic characters are similar among related taxa. Within the genus *Sillago*, differences among some nominal species are generally small and restricted to a few characters. In this study, a new species of *Sillago* (*Sillago shaoi* sp. nov.) was described using morphology and genetic analysis of DNA barcoding. The morphological results differentiated *S. shaoi* sp. nov. from eight other *Sillago* spp. Genetic analysis verified both the validity of the current taxonomy and the relationships between the new species and other *Sillago* species. *S. shaoi* sp. nov. formed a monophyletic group as a distinct phylogenetic species and showed strong genetic divergence from others. The present study also revealed that the COI gene was an effective molecular marker for identifying *Sillago* species.

**Key words:** Sillaginidae, New species, *Sillago shaoi* sp. nov., Taiwan Strait, DNA barcoding.

### BACKGROUND

The family Sillaginidae Richardson, 1846 (Order Perciformes), commonly known as sand whittings or sand borers (McKay 1992), are small to moderate in size and primarily inhabit inshore waters with sandy substrate or estuarine areas of rivers throughout the Indo-West Pacific (McKay 1985; Johnson 1993; Nelson 2006). At present, the family comprises 34 described species belonging to three genera, *Sillago* Cuvier 1817, *Sillaginopsis* Gill 1861, and *Sillaginodes* Gill 1862 (Greenwood et al. 1966; McKay 1992; Nelson 2006; Kaga et al. 2010; Gao et al. 2011 and Golani et al. 2014). Three recently identified species of *Sillago*: *Sillago caudicula* Kaga, Imamura & Nakaya 2010; *Sillago sinica* Gao and Xue 2011; and *Sillago suzezensis* Golani, Fricke & Tikochinski 2014 were wrongly assigned to *Sillago sihama*.

*Sillago sihama*, Forsskål 1775, the senior discovered Sillaginids which inhabits in various

habitats across Indo-West Pacific (IWP, McKay 1992). In fact, accumulating studies suggested widespread species could include cryptic species which could not easily be distinguished by morphological features (Bucciarelli et al. 2002; Kimura et al. 2007; Liu et al. 2013). One of the characters commonly used to identify *Sillago* species was its swim bladder. McKay (1985) reported three subgenera of the genus *Sillago*: *Sillaginopodys* Fowler 1933 (swim bladder reduced, no duct-like process); *Sillago* Cuvier 1817 (swim bladder divided posteriorly into two tapering extensions, duct-like process present); and *Parasillago* (swim bladder with a single posterior extension and the duct-like process) and described four initial species of subgenus *Sillago* (*Sillago* (*Sillago*) *intermedius* Wongratana, 1977; *Sillago* (*Sillago*) *megacephalus* Lin, 1933; *Sillago* (*Sillago*) *parvisquamis* Gill, 1861 and *Sillago* (*Sillago*) *sihama* Forsskål, 1775). Recently, three new *Sillago* species (*S. caudicula*, *S. sinica* and

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*S. suezensis*) were described. The presence of two posterior extensions of the swim bladder observed in three new *Sillago* species suggested that they should belong to the subgenus *Sillago*. In addition, a redescription of *Sillago indica* McKay, Dutt & Sujatha, 1985, reassigned *S. indica* to the subgenus *Sillago* (Kaga and Ho 2012). This subgenera-grading system was very useful in *Sillago* classification and phylogenetic analysis. However, the swim bladders of some sibling species (e.g. *S. sihama* and *S. indica*) were very similar, this made the identification of these species extremely difficult.

While undertaking a taxonomic review of the genus *Sillago* in the coast of China, we had an opportunity to examine forty four specimens of the family collected from the coast of Taiwan Strait, Northwest Pacific Ocean. Based on the morphological characteristics, those specimens were assigned to *S. sihama*; particularly, their swim bladders were very similar to those of *S. sihama* (McKay 1992: Fig. 130, Type Queensland). However, a high mean genetic distance was found between the samples from Taiwan Strait and *S. sihama* collected from coast of China based on COI gene sequences. In addition, morphological evidences indicated that they belonged to a previously unrecognized species. In the present study, we employed morphological methods and DNA barcoding to identify the new *Sillago* species, and reconstructed the relationships of the *Sillago*

species in this region. Our results confirmed the genetic distinction of the existing *Sillago* species and invoked the possibility of the additional species.

## MATERIALS AND METHODS

The unidentified *Sillago* species was collected from the coast of Taiwan Strait, more precisely, in Xiamen (Fujian, 16 individuals), Dongshan (Fujian, 25 individuals), and Changhua (Taiwan, 3 individuals) on December 2013, April 2014, and July 2014, respectively (Fig. 1). All specimens were deposited at the Fishery Ecology & Marine Biodiversity Laboratory, Fishery College, Zhejiang Ocean University (Zhoushan).

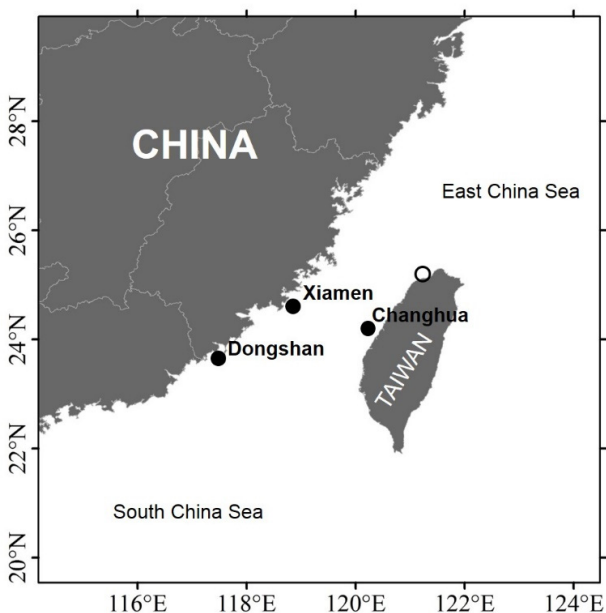
In this study, the recorded eight *Sillago* species with two posterior extensions of the swim bladder were referenced and compared to assign the new *Sillago* species (Table 1). Considered all recorded *Sillago* species in China, twelve *Sillago* taxa were used for comparison.

### Morphological analysis

The genus and species classification followed McKay (1985), unless otherwise noted. The terminology of appendages of the swim bladder followed Shao et al. (1986) and Kaga and Ho (2012). In the descriptive section, the data of the holotypes were given first, while those of the paratypes followed in parentheses. General abbreviations used in this paper were: SL, standard length; HL, head length; D, the number of dorsal fin rays; A, the number of anal fin rays; V, the number of ventral fin rays; P, the number of pectoral fin rays; and C, the number of caudal fin rays. All measurements were made with dial calipers and dividers to the nearest 0.1 mm. The definition of the modified vertebrae followed McKay (1992). Gill rakers and swim bladders were examined in dissected paratypes.

### Genetic analysis

To analyze genetic differences between this new species and other *Sillago* spp., mitochondrial (mt) DNA cytochrome oxidase subunit I (COI) fragments of *Sillago* spp. were amplified based on the method of Ward et al. (2005). Genomic DNA extraction and polymerase chain reaction (PCR) followed the protocols of Gao et al. (2011). Sequences were checked and aligned using



**Fig. 1.** Sampling sites of *Sillago shaoi* sp. nov. The open circle represents the Tamsui River estuary.

DNASTAR software (DNASTAR Inc., Madison, WI, USA) and MEGA 5.0 (Tamura et al. 2011) was used to analyze the sequences, reckon the pairwise genetic distances and construct a neighbor-joining (NJ) tree under the Kimura 2-parameter (K2P) model. Thirty-five COI sequences obtained in the present study were submitted to GenBank with the following accession numbers: *S. shaoi* sp. nov. (KU051872-KU051875, KU051879, KU051880 and KU051886-KU051889), *S. sinica* (KU052012, KU052017, KU052023, KU052025 and KU052029), *S. japonica* (KU051932-KU051935), *S. aeolus* (KU051738, KU051739, KU051747, KU051751 and KU051755), *S. ingenuua* (KU051978-KU051980), *S. asiatica* (KU051912, KU051915 and KU051917) and *S. sihama* (KU051813, KU051819, KU051857, KU051803 and KU051881). Fourteen COI sequences were obtained from GenBank with the following accession numbers: *S. parvisquamis* (HQ389247-HQ389249), *S. chondropus* (JF494511-JF494514), *S. suezensis* (FJ155362-FJ15536), and *S. indica* (KM250229-KM250232). *Sillaginodes punctata* was selected as outgroup for genetic analyses based on a previous phylogenetic hypotheses of Gao et al. (2011).

**RESULTS**

**Taxonomy**

Family Sillaginidae Richardson, 1846  
Genus *Sillago* Cuvier, 1817

***Sillago (Sillago) shaoi* Gao and Xiao, sp. nov.**

urn:lsid:zoobank.org:act:19B9174E-23EE-4A55-9ED1-4E0C9FDCE2D9

(Fig. 2)

**Holotype:** FEMBL150001, 182.2 mm SL, coastal area of Xiamen, Fujian Province, China, collected by Yuan Li, December 2013.

**Paratypes:** FEMBL150002-150016, 15 individuals, 173.0-203.3 mm SL, collection data same as for holotype; FEMBL150017-150019, 3 individuals, 146.2-167.6 mm SL, coastal area of Changhua County, Taiwan, collected by Shih-Chieh Shen, July 2014; FEMBL150020-150039, 20 individuals, 182.0-216.0 mm SL, coastal area of Dongshan, Fujian Province, China, collected by Yuan Li, April 2014. All specimens were deposited at the Fishery Ecology & Marine Biodiversity Laboratory, Fishery College, Zhejiang Ocean University (Zhoushan, China).

**Etymology:** The specific name “shaoi” was named in honor of the taxonomist Dr. Kwang-

**Table 1.** Comparison of *Sillago shaoi* sp. nov. and eight other species of *Sillago* with two posterior extensions of the swim bladder

	<i>S. shaoi</i> sp. nov. <sup>a</sup>	<i>S. intermedius</i> <sup>b</sup>	<i>S. megacephalus</i> <sup>b</sup>	<i>S. parvisquamis</i> <sup>b,c</sup>
Dorsal fins	XI,I,20-22	XI,I,21-22	XI,I,22	XII-XIII,I,20-22
Anal fin	II,21-22	II,21-22	II,23	II,22-24
Scales in lateral line	70-73	67-70	70	79-84
Scales above/below lateral line	5-6/10-12	6-7/8-9	5/10-11	7/11-12
Gill rakers first arch	3-4/5-6	—	—	1-2/7-9
Vertebrae	35	34	—	39-40
Blotches	Absent	Present	Absent	Absent
HL/SL (%)	26.1-31.0	30.0-31.0	33.0	25.9-27.7

	<i>S. sihama</i> <sup>a,b</sup>	<i>S. caudicula</i> <sup>d</sup>	<i>S. sinica</i> <sup>e</sup>	<i>S. suezensis</i> <sup>f</sup>	<i>S. indica</i> <sup>g</sup>
Dorsal fins	XI,I,20-23	XI,I,22-23	X-XI,I,20-22	X-XII,I,19-22	X-XI,I,20-22
Anal fin	II,21-23	II, 23-24	II, 21-23	II,18-22	II,21-23
Scales in lateral line	68-72	71	75-79	63-74	68-71
Scales above/below lateral line	5-6/10-12	5/11	5-6/9-11	—	5-6/10-12
Gill rakers first arch	3/8-9	4/11	2-4/6-8	3-4/8-10	3-4/7-8
Vertebrae	34	35-36	37-39	34	33-35
Blotches	Absent	Present	Absent	Absent	Absent
HL/SL (%)	24.0-30.0	29.0-30.1	24.7-29.8	26.6-27.0	27.5-32.4

Notes: a, in this study; b, McKay 1985; c, Xue 2010; d, Kaga et al. 2010; e, Gao et al. 2011; f, Golani et al. 2014; g, Kaga and Ho 2012.

Tsao Shao for his remarkable contribution in classification of fishes.

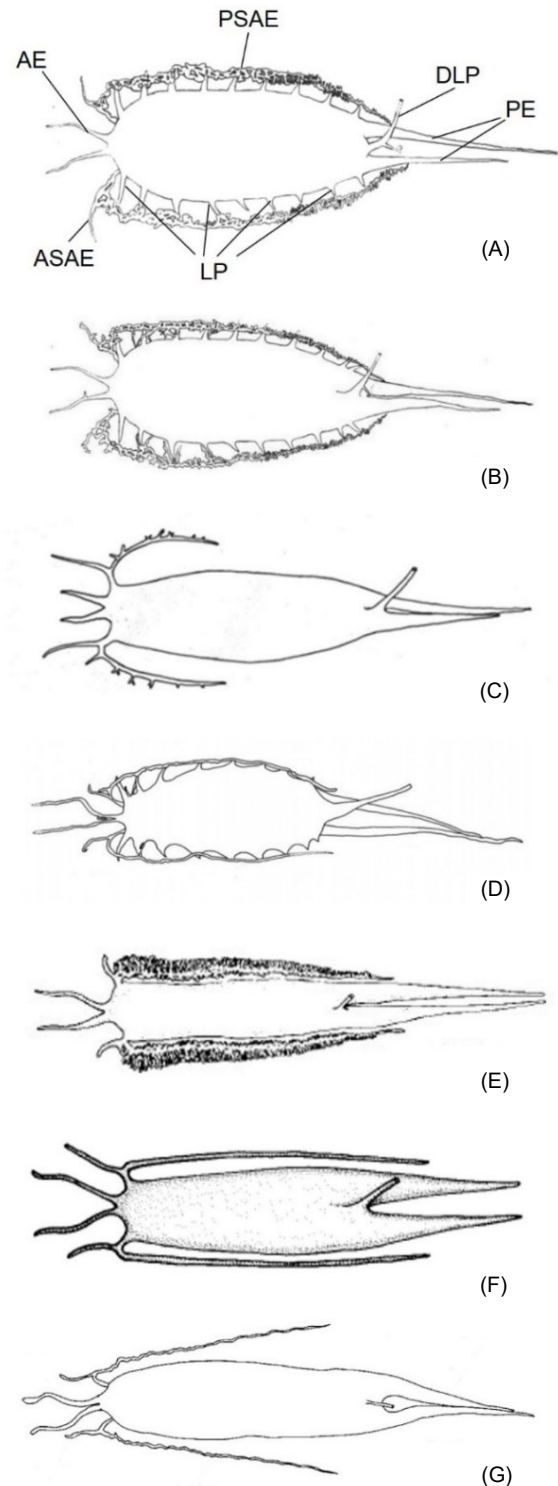
**Diagnosis:** A new species of the genus *Sillago* and differs from other *Sillago* species with a relatively larger body size; the 1st dorsal fin *S. shaoi* sp. nov. has XI (not to XII), the 2nd dorsal fin with I and 20-22 soft fin rays; 21-22 soft anal fin rays; scales in lateral line 70-73, scales above lateral line 5 or 6; gill rakers 3-4+5-6; vertebra: abdominal 13-14 (mostly 14), modified 4-6 (mostly 5), caudal 15-17 (mostly 16), and total 35. Apparent differences among shape of swim bladders of the 9 *Sillago* species (with two posterior extensions of the swim bladder) showed *S. shaoi* sp. nov. had a similar swim bladder with *S. sihama* but differed in detail (Fig. 3). Genetic analysis also showed strong interspecific differentiation among the six *Sillago* species with two posterior extensions of the swim bladder and other five *Sillago* species in coastal waters of China (Table 2).

**Description:** General body features were shown in figure 2. Counts and measurements were given in table 3. Description was based on the holotype, data of paratypes were shown in parentheses when variation was recognized between holotype and paratypes.

Body elongate, anterior slightly pyramidal, posterior cylindrical; anterodorsal profile smooth. Body depth 17.1% (13.4-17.9%) in SL. Head large, length 28.3% (26.1-31.0%) in SL. Snout long, 46.6% (41.8-50.2%) of HL. Eye moderate, its margin slightly covered with adipose eyelid, diameter 21.3% (14.9-24.1%) of HL. Interorbital region flat, interorbital width 27.0% (17.5-27.0%) of HL. Nostrils situated anterior to upper margin of eye; posterior margin of anterior nostril with single anteriorly directed flap; posterior nostril lacking



**Fig. 2.** *Sillago shaoi* Gao and Xiao, sp. nov., FEMBL150001, holotype, 182.2 mm SL, Zhoushan, China.



**Fig. 3.** Swim bladders of 7 *Sillago* species. (A) *S. shaoi* sp. nov., (B) *S. sihama*, (C) *S. sinica*, (D) *S. indica* (Kaga and Ho 2012), (E) *S. parvisquamis* (McKay 1992), (F) *S. intermedius* (McKay 1992), (G) *S. caudicula* (Kaga et al. 2010). AE, anterior extension; ASAE, anterior sub-extension of anterolateral extension; PSAE, posterior sub-extension of anterolateral extension; LP, lateral processes; DLP, duct-like process; PE, posterior extension.

flap. Mouth small, terminal, anterior tip of upper jaw situated at almost same position as tip of lower jaw. Upper jaw with small canines forming a wide tooth band becoming narrower posteriorly. Lower jaw with small canines, forming tooth band anteriorly, width same as upper jaw tooth band, tooth band gradually becoming narrower posteriorly, ending in one row. Palatine and tongue toothless. Vomer with three to four rows of canine teeth. Posterior margin of preopercle slightly serrated. Gill aperture large, lateral, extending to ventral side of head, stopping at middle bottom of opercle. Gill rakers on first arch pointed but short. Caudal peduncle short, depth of caudal peduncle 71.7% (56.6-88.9%) of length of caudal peduncle.

Body scales deciduous, size moderate, ctenoid except for those on prenasal area, which were cycloid. Cheek scales deciduous, cycloid, arranged in about two or three rows. Lower part of pre-opercular-mandibular canal covered with cycloid scales. Pectoral fin base and ventral fin base lacking scales. Lateral line beginning above gill aperture and anterior portion of pectoral fin, extending along curve of dorsal edge to end of

body.

Two separated dorsal fins, 1st dorsal fin XI, higher than 2nd one obviously, origin posterior to top of pectoral fin base, composed of spines, gradually shortening. Fin membrane with dense black spots. Base of 2nd dorsal fin long, composed of 1 spine and 20 (20-22) soft rays, originating mid-body, and not extending to caudal fin origin when placed flat. Origins of anal fin slightly posterior to cloacal pore, with II + 21 (21-22), not extending to caudal fin origin when placed flat. Pectoral fin 16 (16-18), slender. Two separate ventral fins broad, I+5, roughly triangular, and shorter than pectoral fin. Comparisons of meristic characters of nine *Sillago* species with two posterior extensions of the swim bladder were shown in table 1.

*Color of fresh specimens:* Upper surface of head dark brown and trunk bright-brown, grading to silver on abdomen. Dorsal side of snout brownish-gray. Cheek slightly silver, with black dots massed on the anterior inferior part of eyes. A wide faint stripe composed of tiny black dots on skin sometimes present, from opercular to caudal peduncle. Dorsal fins yellowish-hyaline, small dark

**Table 2.** Pairwise genetic distances (K2P) between 11 *Sillago* species

	<i>S. aeolus</i>	<i>S. chondropus</i>	<i>S. japonica</i>	<i>S. suzeensis</i>	<i>S. ingenuua</i>
<i>S. aeolus</i>					
<i>S. chondropus</i>	0.219				
<i>S. japonica</i>	0.220	0.207			
<i>S. suzeensis</i>	0.181	0.239	0.203		
<i>S. ingenuua</i>	0.198	0.223	0.203	0.222	
<i>S. parvisquamis</i>	0.205	0.227	0.226	0.193	0.190
<i>S. asiatica</i>	0.181	0.231	0.220	0.194	0.217
<i>S. indica</i>	0.209	0.238	0.218	0.084	0.216
<i>S. sinica</i>	0.194	0.208	0.209	0.211	0.204
<i>S. sihama</i>	0.182	0.211	0.202	0.177	0.197
<i>S. shaoi</i> sp. nov.	0.176	0.234	0.212	0.213	0.203

	<i>S. parvisquamis</i>	<i>S. asiatica</i>	<i>S. indica</i>	<i>S. sinica</i>	<i>S. sihama</i>
<i>S. aeolus</i>					
<i>S. chondropus</i>					
<i>S. japonica</i>					
<i>S. suzeensis</i>					
<i>S. ingenuua</i>					
<i>S. parvisquamis</i>					
<i>S. asiatica</i>	0.226				
<i>S. indica</i>	0.202	0.226			
<i>S. sinica</i>	0.168	0.209	0.214		
<i>S. sihama</i>	0.210	0.176	0.172	0.211	
<i>S. shaoi</i> sp. nov.	0.152	0.211	0.224	0.123	0.196

dense spots on fin membrane. Pectoral, ventral, and anal fins yellowish with dark spots; caudal fin yellowish dusky with a black margin and grayish-brown margin posteriorly, lobes usually broadly truncated posteriorly.

**Swim bladder:** Swim bladder large. Two anterior extensions diverging to terminate on either side of the basioccipital above the auditory capsule. Two posterior tapering extensions of the swim bladder penetrating into the caudal region, one usually longer than the other. Two anterolateral extensions originate anteriorly, each branch into anterior and posterior sub-extensions: the anterior one comprising a short, simple blind tubule and the posterior sub-extensions were

kinked, long and complex, extending along the abdominal wall ventral to the peritoneum to the base of the posterior extensions, respectively, tangent but not interconnected. A single duct-like process originating from ventral surface of swim bladder extending to the urogenital opening and a sub-extension was present connecting with a sanguineous vesicle close to vertebra, with unknown function. Eight or nine lateral processes extending from entire lateral surface of main body of swim bladder, anterior three or four stout and horn-like, posterior five or six rather small and triangular in shape.

**Habitat:** Habitat is similar to *S. sihama* in nearshore areas and frequently entering estuaries

**Table 3.** Morphometric measurements for type specimens of *Sillago shaoi* sp. nov.

Morphometric measurements (mm) and counts	Holotype FEMBL150001	Paratypes FEMBL150002-150039 (n = 38)
Total weight (TW, g)	65.6	62.8-113.9
Total length (TL)	201.8	196.6-265.0
Standard length (SL)	182.2	173.0-216.0
Head length (HL)	51.6	48.1-60.2
Snout length (SL)	24.0	21.8-27.8
Eye diameter (ED)	10.9	7.6-12.9
Interorbital width (IW)	13.9	9.1-14.2
Postorbital length (PL)	20.3	16.2-26.2
Body depth (BD)	31.2	27.5-34.2
Body width (BW)	25.6	21.4-26.5
Length of caudal peduncle (LCP)	20.5	15.2-23.6
Depth of caudal peduncle (DCP)	14.7	12.4-15.1
Base of the 1st dorsal fin	39.2	31.0-46.0
Base of the 2nd dorsal fin	65.0	55.5-78.4
Base of the anal fin	63.4	59.5-75.0
Pectoral fin length	32.3	30.0-39.4
Ventral fin length	28.1	21.7-28.8
D	XI, I+20	XI, I+20-22
P	16	16-18
V	I+5	I+5
A	II+21	II+21-22
C	17	16-18
Gill rakers first arch	3+6	3-4+5-6
Vertebrae	35	35
Scales on lateral line	72	70-73
Scales above/below lateral line	5/11	5-6/10-12
As % of SL		
Body depth (BD)	17.1	13.4-17.9
Head length (HL)	28.3	26.1-31.0
Length of caudal peduncle (LCP)	11.3	8.2-12.4
As % of HL		
Eye diameter (ED)	21.1	14.9-24.1
Interorbital width (IW)	26.9	17.5-27.0
Snout length (SL)	46.5	41.8-50.2
Postorbital length (PL)	39.3	31.4-43.0
DCP/LCP	71.7	56.6-88.9

for considerable periods. It is common along the beaches, sandbars and mangrove creeks with sandy substrate. In depths ranging from 0 to 20 m, and frequently captured by trawling vessels.

**Distribution:** *Sillago shaoi* sp. nov. was presently only found in coastal waters of the Taiwan Strait, larvae and juveniles of this species were found in the estuarine area of the Tamsui River (25.20°N, 121.38°E) in northern Taiwan (provided by Dr. Kwang-Tsao Shao) (Fig. 1).

## Comparison

According to the subgenera-grading system in genus *Sillago* proposed by McKay (1985), we used the characters of swim bladder, especially, the number of the posterior extensions to divide *Sillago* into several categories; and confirmed the validity of a new *Sillago* species with two posterior extensions by comparison-elimination other species in the same category. Among the eight known members of *Sillago* with two posterior extensions, we could easily distinguished *S. shaoi* sp. nov. from *S. intermedius* and *S. caudicula* by the body coloration (dusky black blotches were present on the body of *S. intermedius* and *S. caudicula*), from *S. parvisquamis* and *S. sinica* by the dusky spots on the second dorsal fin membranes (five or six rows in *S. parvisquamis* and three or four rows in *S. sinica*). Empirically, we could also distinguish *S. shaoi* sp. nov. from *S. sihama*, *S. indica* and *S. suezensis* by the coloration of anal fin (the anal fin of *S. shaoi* sp. nov. was usually yellowish, the anal fin of *S. indica* was yellowish-brown, but the anal fin of *S. sihama* and *S. suezensis* were hyaline; on the other hand, there were more black dots on skin and fins of *S. indica* than on *S. shaoi* sp. nov. when fresh).

Moreover, by the primary diagnostic features (Table 1), *S. shaoi* sp. nov. was easily distinguishable from others by the following: *S. megacephalus* by having a smaller head (26.1-31.0% SL in *S. shaoi* sp. nov. vs. 33.0% in *S. megacephalus*) and soft rays in anal fin (21-22 in *S. shaoi* sp. nov. vs. 23 in *S. megacephalus*), from *S. intermedius*, *S. parvisquamis*, *S. sihama*, *S. suezensis* and *S. sinica* by having 35 total vertebrae (34 in *S. intermedius*, *S. sihama*, *S. suezensis*; 39-40 in *S. parvisquamis* and 37-39 in *S. sinica*), from *S. parvisquamis* and *S. sinica* by having 70-73 scales on lateral line (79-84 in *S. parvisquamis* and 75-79 in *S. sinica*), from *S. indica*, *S. suezensis* and *S. sihama* by having 3-4/5-6 gill rakers (3-4/7-8 in *S. indica*, 3-4/8-10 in

*S. suezensis* and 3/8-9 in *S. sihama*), and from *S. caudicula* by gill rakers (4/11 in *S. caudicula*) and soft rays in anal fin rays (23-24 in *S. caudicula*) (Table 1).

As for the shape of swim bladder (Fig. 3), *S. suezensis* was always controversial (Kaga 2013). Judging from its original description based on the holotype: the figures of the swim bladder (Golani et al. 2014: 418, Fig. 4, A-C) were stylized; lacked the details of those provided by McKay (1985, 1992) and Kaga and Ho (2012). However, the sequences of *S. suezensis* (Mediterranean population) and *S. sihama* (Hong Kong and southern Red Sea populations) showed a strong genetic divergence (Tikochinski et al. 2013). Here, those sequences were also cited to verify authenticity of *S. shaoi* sp. nov. and dismissed *S. suezensis* (Fig. 4).

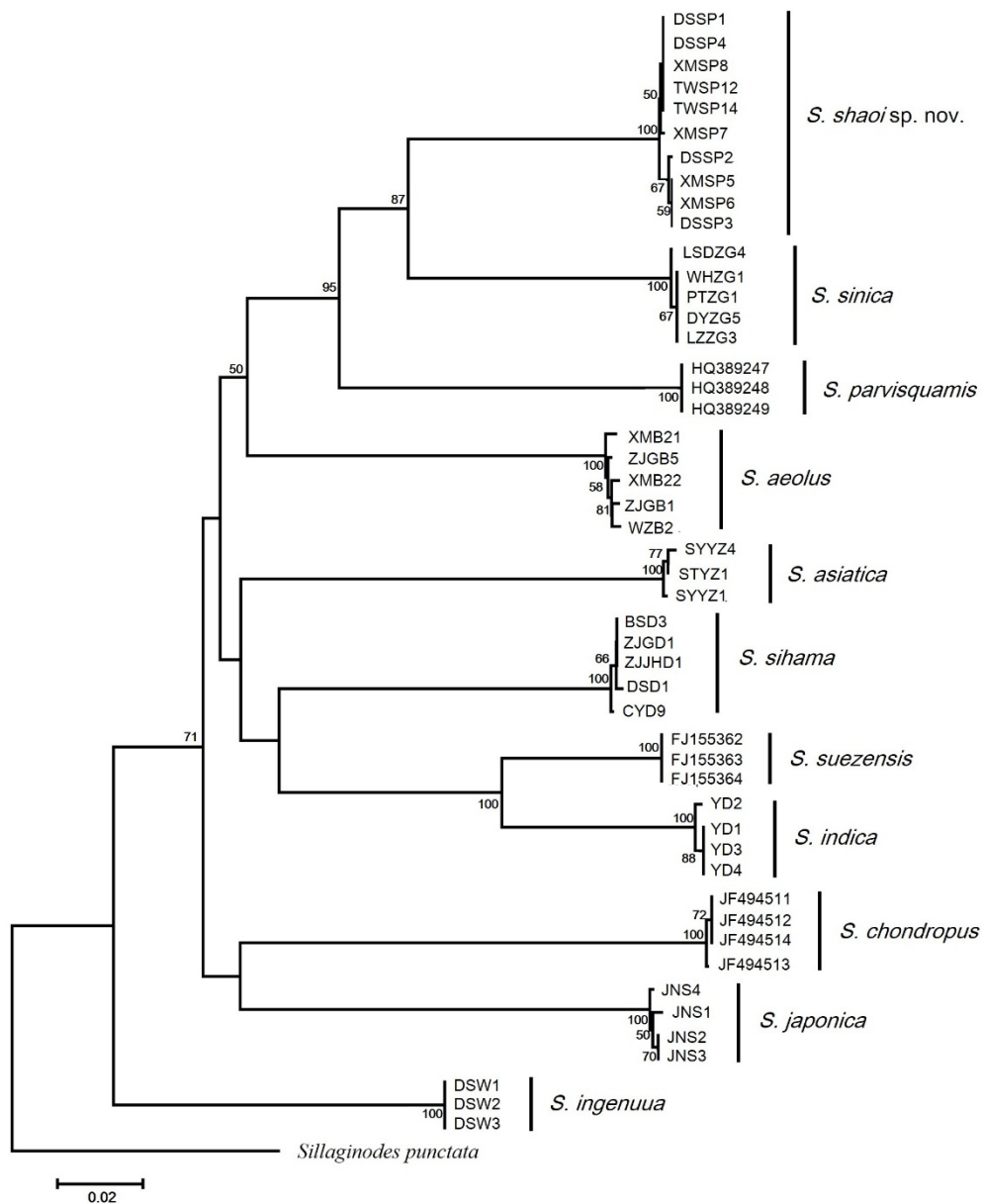
*Sillago sihama* was considered as having a wide Indo-Pacific distribution and consisting of more than one taxon. McKay (1992: 59, Fig. 130) described two swim bladder patterns of *S. sihama* in the FAO Catalogue based on a Red Sea specimen and a Queensland specimen with markedly different shape and concomitant divergence. The swim bladder of *S. shaoi* sp. nov. was very similar to that of *S. sihama* but there were still some differences: the roots of two posterior extensions in *S. shaoi* sp. nov. were non-adjacent, two posterior extensions were not well-knit, in its natural state, and there was a lacuna between the two posterior extensions; the origin of the duct-like process was at the terminal of swim bladder and between the roots of two posterior extensions. However, on the swim bladder of *S. sihama*, the roots of two posterior extensions were adjacent and two posterior extensions were in close; the origin of the duct-like process was anterior to the terminus of swim bladder and anterior to the joint of roots of two posterior extensions. Swim bladder of *S. indica* had the same framework with *S. shaoi* sp. nov. excepting the thin-simple anterolateral extensions (vs *S. shaoi* sp. nov., anterolateral extensions were kinky, long and complicated). *S. shaoi* could also be easily distinguished from *S. intermedius* and *S. caudicula* by those swim bladder with simple anterolateral extensions; *S. parvisquamis* stood out with the strongest anterolateral extensions in comparison with the others (Fig. 3).

Recently, Gao et al. (2011) described a new *Sillago* species - *S. sinica* from the East China Sea without describing the shape of the swim bladder, the most important characteristic for identifying Sillaginids. Here, the swim bladder of



*S. sinica* was described for the first time and its subgeneric status was reassigned. Compared with other *Sillago* species with two posterior extensions of the swim bladder, *S. sinica* was unique (Fig. 3). Two anterior extensions extending forward and diverging to terminate on each side of the basioccipital above the auditory capsule. Two posterior tapering extensions of the swim bladder penetrating into the caudal region, one usually longer than the other. Two anterolateral extensions

commencing anteriorly, each branch into anterior and posterior sub-extensions: the anterior one comprising a simple blind tubule, extending forward and usually slightly longer than anterior extensions; the posterior sub-extensions were unique with some stunted blind tubule, unilateral and outward; the posterior sub-extensions extending along the abdominal and about one-third of the body of swim bladder in length; a single duct-like process present.



**Fig. 4.** Neighbor-joining (NJ) tree for cytochrome oxidase subunit I (COI) gene sequences of 11 species of Sillaginidae. The NJ tree was constructed under the K2P model using *Sillaginodes punctata* as outgroup. Bootstrap support values of > 50% from 1000 replicates are shown.



## Genetic analysis of the COI gene

Forty-nine specimens of 11 *Sillago* species were used in the genetic analysis. After sequence alignment, a 583 bp fragment of the COI gene was obtained. There were no indels/insertions, and 207 variable sites were observed. Pairwise genetic distances (K2P) were shown in table 2. Genetic distances among *Sillago* species ranged from 0.123 to 0.239. The NJ tree based on COI gene sequences (Fig. 4) revealed that all previously recognized and the newly discovered *S. shaoi* sp. nov. individuals formed monophyletic groups. The new species *S. shaoi* sp. nov. and *S. sinica* were clustered firstly. Furthermore, a strong genetic divergence was detected between *S. shaoi* sp. nov. and its plesiomorphic species - *S. sihama*.

## Key to *Sillago* species (swim bladder with two posterior extensions)

1. Body with dark blotches or spots ..... 2
  - Body without dark blotches or spots ..... 3
2. Anal fin rays  $\geq$  23; vertebrae more than 34 ..... *S. caudicula*
  - Anal fin rays less than 23; vertebrae 34 ..... *S. intermedius*
3. HL/SL less than 33% ..... 4
  - HL/SL is 33% ..... *S. megacephalus*
4. Second dorsal fin membrane with many rows of clear dusky spots ..... 5
  - Second dorsal fin membrane without any row of dusky spots ..... 6
5. Second dorsal fin with 5 or 6 rows of dusky spots along rays ..... *S. parvisquamis*
  - Second dorsal fin with 3 or 4 rows of dusky spots along rays ..... *S. sinica*
6. Anal fin membrane hyaline ..... 7
  - Anal fin membrane usually with spots ..... 8
7. Preopercle and most of the opercle without scales .....
  - ..... *S. suezensis*
  - Preopercle and most of the opercle with scales .....
    - ..... *S. sihama*
8. Gill rakers on the first arch 3-4/7-8; anterolateral extensions of swim bladder thin ..... *S. indica*
  - Gill rakers on the first arch 3-4/5-6; anterolateral extensions of swim bladder strong ..... *S. shaoi* sp. nov.

## DISCUSSION

This study described a new species *Sillago shaoi* sp. nov., defined by morphological and DNA barcoding methods. Results of the genetic analysis were in agreement with the morphological data. All *Sillago* species were monophyletic with 100% bootstrap values, *S. shaoi* sp. nov. was grouped with *S. sinica*, and shared a significant genetic distance with *S. sihama* in the COI genetic analysis. Genetic distances between *S. shaoi*

sp. nov. and other *Sillago* species ranged from 0.123 to 0.234, which indicated that the COI gene used as a barcode is an effective tool to identify *Sillago* species. The results also confirmed the distinctiveness of the new species.

Based on genetic comparisons, and combining the previous records, we presented a synopsis of family Sillaginidae from the coast of China. Except *Sillago shaoi*, there were a total number of thirty four species in this family and twelve of them distributing in the coast of China: *S. aeolus*, *S. ingenuua*, *S. sihama*, *S. chondropus*, *S. ciliata*, *S. parvisquamis*, *S. sinica*, *S. microps*, *S. japonica*, *S. boutani*, *S. asiatica* and *S. megacephalus*. Among them, *S. boutani* and *S. megacephalus* were only described based on the holotypes and lack supporting studies (McKay 1985). Shao et al. (1986) reported eight of them distributing in Taiwan (*S. aeolus*, *S. ingenuua*, *S. sihama*, *S. chondropus*, *S. parvisquamis*, *S. microps*, *S. japonica* and *S. asiatica*) and *S. microps* was endemic species. Moreover, *S. ciliate* which distributed in the east coast of Australia according to the FAO Catalogue (McKay 1992) was reported in the coast of Kaohsiung based on the fish database of Taiwan. Therefore, a comprehensive survey on the family Sillaginidae in the coast of China need to be done in the future.

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