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Description and DNA Barcoding of a New *Sillago* Species, *Sillago sinica* (Perciformes: Sillaginidae), from Coastal Waters of China

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Tian-Xiang Gao, Dong-Ping Ji, Yong-Shuang Xiao, Tai-Qiang Xue, Takashi Yanagimoto, and Takumi Setoguma (2011) Description and DNA barcoding of a new *Sillago* species, *Sillago sinica* (Perciformes: Sillaginidae), from coastal waters of China. *Zoological Studies* **50**(2): 254-263. A new species of the genus *Sillago*, Chinese sillago, *Sillago sinica* sp. nov., was described using morphological methods and phylogenetic analysis of DNA barcoding of 53 specimens collected from the East China Sea (Wenzhou), Yellow Sea (Qingdao), and Bohai Sea (Dongying, China). Results of the morphological analysis (such as vertebrate counts, otoliths, etc.) showed that significant differentiation existed between *S. sinica* sp. nov. and 5 other *Sillago* spp. The mitochondrial DNA cytochrome oxidase subunit I (COI) gene was used as a DNA barcode to clarify the systematics of the genus *Sillago*. Results of the phylogenetic analysis showed that *S. sinica* sp. nov. formed a monophyletic group as a distinct phylogenetic species. It was also suggested that the COI gene is an effective molecular marker for identifying *Sillago* species. http://zoolstud.sinica.edu.tw/Journals/50.2/254.pdf

Key words: Sillaginidae, New species, Chinese sillago, *Sillago sinica*, COI.

Fish belonging to the family Sillaginidae are small to moderate in size and primarily inhabit inshore waters with sandy substrate or estuarine areas of rivers. Some species enter estuaries and even penetrate fresh water for considerable periods, despite the absence of renal corpuscles in the kidney (Nadkarni 1963). The family is widely distributed throughout the Indian and western Pacific Oceans (McKay 1992). At present, the family includes 31 species belonging to 3 genera: *Sillago* Cuvier, 1817; *Sillaginopsis* Gill, 1861; and *Sillaginodes* Gill, 1862, with 29 species in the genus *Sillago* (Fishbase 2010). The taxonomy of the family is approaching stability, and only a few undescribed species likely remain (McKay 1985 1989). Three valid species of *Sillago* (*S. sihama* Forsskål, 1775; *S. japonica* Temminck and Schlegel, 1843; and *S. aeolus* Jordan and Evermann, 1902) were recorded in coastal waters of China (Cheng and Zheng 1987). There is the 4th species, *S. parvisquamis* Gill, 1861, recorded in both Japan and Korea (Sano and Mochizuki 1984, Nakabo 2000, Kim 2005). In addition to those 4 species, there are 4 other species of *Sillago* in Taiwan: *S. asiatica* McKay, 1983; *S. chondropus* Bleeker, 1849; *S. ingenuua* McKay, 1985; and *S. microps* McKay, 1985 (Shao et al. 1986, Shen et al. 1993).

Sillaginids are easily identified as a family due to the great similarity of shape and general uniformity of coloration. The fish have an elongate body, a conical snout, long dorsal and anal fins, and a horizontal lower portion of the preopercle. The body is covered with small- or moderate-

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sized ctenoid scales, and the cheek scales are cycloid or ctenoid. There are 2 dorsal fins, the 1st with X-XIII slender spines, the 2nd with I slender leading spine and 16-27 soft rays. The similar morphological characters; however, have led to much confusion in their specific identification, and many true species may have been concealed in the synonymy of wide-ranging species (Shao and Chang 1978, Shao et al. 1986, McKay 1992).

Species of *Sillago* are widely distributed over inshore areas, and feed on benthic or epibenthic organisms. The white flesh and exceedingly delicate flavor make them esteemed table fish throughout their range (McKay 1992). In the present study, we employed morphological methods and DNA barcoding to identify a new species, *S. sinica* sp. nov., and reconstructed the phylogenetic relationship of the genus *Sillago*. An understanding of the systematics of the genus *Sillago* should provide an important basis for the conservation of species diversity in the family Sillaginidae.

MATERIALS AND METHODS

An unidentified *Sillago* species was collected from an estuarine area of the Feiyun River in Wenzhou (East China Sea, 14 individuals), an estuarine area of the Yellow River in Dongying (Bohai Sea, 28 individuals), and a coastal area in Qingdao, China (Yellow Sea, 11 individuals) in June, Sept., and Oct. 2009, respectively. All specimens were deposited at the Fisheries Ecology Laboratory, Fisheries College, Ocean Univ. of China (Qingdao).

Morphological analysis

In this study, the species was identified as a new species of Perciformes in coastal waters of China and was compared to 5 other species of the genus *Sillago*: *S. bassensis* Cuvier, 1829, *S. parvisquamis*, *S. japonica*, *S. sihama*, and *S. aeolus*.

All counts, measurements, and general terminology followed Hubbs and Lagler (1947). Measurements were made point to point with dial calipers to 0.01 mm. Definitions of the character states in this paper follow those of Anderson (1994). Abbreviations used in this paper are: BL, body 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.

After measurements were taken, both sagittae were removed from each specimen following CCAMLR (2006) and placed in 1.5-ml plastic tubes containing distilled water. After ultrasonic cleaning, the otoliths were baked in an oven for 24 h at 50°C until a constant mass and weight to the nearest 0.01 mg were achieved. Lateral-view digital images of otoliths were taken with a Nikon SMZ800 microscope equipped with a Nikon digital sight DS-Fi1 (Tokyo, Japan). Since there were no differences in morphological characters between right and left otoliths, only left otoliths were used in the study.

Phylogenetic analysis

To analyze genetic differences between *S. sinica* sp. nov. 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 was extracted from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method (Sambrook et al. 1989). Each polymerase chain reaction (PCR) was performed in a volume of 25 μl containing 1 μl template DNA, 2.5 μl of 10 × PCR buffer, 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 0.2 mmol/L of each primer, and 1.25 units of *Taq* DNA polymerase in an Eppendorf Mastercycler 5333 (Eppendorf, Hamburg, Germany). The COI gene fragment was amplified with primers L5956-COI (5'-CACAAAGACATTGGCACCCT-3') and H6558-COI (5'-CCTCCTGCAGGGTCAAA GAA-3') (Inoue et al 2001). Initial denaturation was for 3 min at 94°C, followed by 30 cycles of 45 s at 94°C for denaturation, 40 s at 52°C for annealing, and 45 s at 72°C for extension, with a final extension at 72°C for 15 min. All PCR sets included a negative control reaction tube in which all reagents were included except the template DNA. The PCR products were separated on a 1.5% agarose gel and purified with the BioDev Gel Extraction System B (BioDev Technology, Beijing, China). The purified products were sequenced using a BigDye Terminator cycle sequencing kit v2.0 (Applied Biosystems, Foster City, CA, USA), and sequencing was conducted on an ABI Prism 3730 automatic sequencer (Applied Biosystems) with both forward and reverse primers used for amplification. Lasergene software (Lasergene, Madison, WI, USA) was used for sequence comparison, and MEGA 4.0 (Tamura et al. 2007) was used to analyze the sequences and

construct a Neighbor-joining (NJ) tree under the Kimura 2-parameter (K2P) model. Sixteen COI sequences were obtained from GenBank with the following accession numbers: *S. japonica* (HM131471, HM131472, HM131485, and HM131486), *S. aeolus* (HM131473-HM131476), *S. sihama* (HM131477-HM131480), and *S. bassensis* (HM131481-HM131484). Eighteen COI sequences obtained in the present study were submitted to GenBank with the following accession numbers: *S. sinica* sp. nov. (HQ389239-HQ389245 and HQ389254-HQ389256), *S. sihama* (HQ389246), *S. parvisquamis* (HQ389247-HQ389249), and *S. japonica* (HQ389250-HQ389253). The COI sequences for *Sillaginodes punctatus* used as an outgroup was obtained from GenBank with the accession no. EF609465 (Ward and Holmes 2007).

TAXONOMY

Family Sillaginidae Richardson 1846
Sillago Cuvier 1817
Sillago sinica Gao and Xue, sp. nov.
(Fig. 1)

Holotype: OUC_FEL100348, 122.79 mm SL, estuarine area of Feiyun R. (27°40'N, 120°44'E, East China Sea), near Wenzhou City, Zhejiang Prov., China, collected by Xiao Chen, June 2009.

Paratypes: OUC_FEL100349-100361, 13 individuals, 117.79-157.37 mm SL, collection data same as for holotype; OUC_FEL100362-100388, OUC_FEL100403, 28 individuals, 94.36-134.17 mm SL, estuarine area of Yellow R. (38° 11'N, 118°41'E, Bohai Sea), near Dongying City, Shandong Prov., China, collected by Tianxiang Gao, Sept. 2009; OUC_FEL100390-100399, OUC_FEL100404, 11 individuals, 79.21-137.20 mm SL, coastal area of Qingdao (36° 01'N, 120°27'E, Yellow Sea), Shandong Prov., China, collected by Taiqiang Xue, Oct. 2009. All specimens were deposited at the Fisheries Ecology Laboratory, Fisheries College, Ocean Univ. of China (Qingdao, China).

Etymology: The specific name "*sinica*" is derived from the Latin and refers to all sampling sites in coastal waters of China: the East China Sea, Bohai Sea, and Yellow Sea. The gender is feminine.

Diagnosis: It differs from other *Sillago* species; the 1st dorsal fin *S. sinica* sp. nov. has X or XI (not to XII); scales above lateral line 7 or 8; gill rakers 2-4+6-8; vertebra: abdominal 13-17 (mostly 15), modified 0-4 (mostly 2), caudal 19-24 (mostly 21), and total 37-39 (mostly 38). We could also see apparent differences among pictures of otoliths of the 6 *Sillago* species with *S. sinica* sp. nov. having a kernel-shaped otolith with a wavy edge (Fig. 3F). Genetic analysis also showed interspecific differentiation among the 6 *Sillago* species.

Description: General body features are shown in figure 1. Morphometric data are given in table 1. HL 24.71%-29.75% of BL. Body elongate, anterior portion slightly pyramidal, posterior portion cylindrical. Anterodorsal profile smooth, gently curving at an angle of 25° to horizontal axis. Body depth 11.26%-18.36% of BL. Snout long, 33.82%-45.10% of HL. Eye small and not concave, with 2 or 3 rows of small ctenoid scales. Eye diameter 15.38%-22.66% of HL, interorbital width 18.97%-28.11% of HL. Both sides of body with a separated nostril in anterior portion of eye. Mouth small, forward position with a flat crack.

Fig. 1. *Sillago sinica* Gao and Xue, sp. nov., OUC_FEL100404, paratype, 137.20 mm SL, Qingdao, China.

Upper jaw slightly prominent; posterior edge of maxillary not extending to bottom edge of eye; lacking jaw teeth. Gill aperture large, lateral, extending to ventral side of head, stopping at middle bottom of opercle. Cheek large, gill rakers short. Caudal peduncle short, depth of caudal peduncle 48.21%-68.38% of length of caudal peduncle.

Body with ctenoid scales, anterior portion larger than posterior portion. Pectoral fin base and ventral fin base lacking scales. Lateral line beginning above gill aperture and anterior portion of pectoral fin, running along curve of dorsal edge to end of body.

Two complete separated dorsal fins; 1st dorsal fin X or XI, higher than 2nd one, beginning behind top of pectoral fin base, composed of spines, 2nd spine longest, others gradually shortening. No spines extending to 2nd dorsal fin when placed flat. Fin membrane with irregular black spots. Base of 2nd dorsal fin long, composed of 1 spine and 20-22 soft rays, starting at middle of body, and not extending to caudal fin origin when placed flat. Origins of anal fin slightly posterior to 2nd dorsal fin, with II + 21 - 23, not extending to caudal fin origin when placed flat. Pectoral fin 15-18, slender. Two separate ventral fins broad, I+5, roughly triangular, and shorter than pectoral fin. Outermost rays with filamentous extensions, and longest may reach anterior end

Table 1. Morphometric measurements for type specimens of *Sillago sinica* sp. nov.

Morphometricmeasurements (mm) and counts	Holotype OUC_FEL100348	Paratypes OUC_FEL100349-100399, 100403, 100404 (*n* = 52)
Total weight (TW)	14.60	3.50-29.80 (13.28)
Total length (TL)	141.48	92.31-174.96 (128.27)
Body length (BL)	122.79	79.21-157.37 (111.61)
Head length (HL)	34.11	21.18-43.18 (30.11)
Snout length (SL)	14.39	7.71-18.45 (12.20)
Eye diameter (ED)	5.83	4.35-7.30 (5.59)
Interorbital width (IW)	7.89	4.76-10.73 (6.86)
Postorbital length	14.67	5.71-20.04 (12.55)
Upper jaw length (UJL)	7.49	4.02-9.15 (6.28)
Body depth (BD)	17.33	10.60-23.82 (17.22)
Body width (BW)	13.41	7.49-19.52 (12.55)
Length of caudal peduncle (LCP)	13.08	8.47-18.38 (13.12)
Depth of caudal peduncle (DCP)	7.22	4.72-10.52 (7.48)
Base of the 1st dorsal fin	22.09	13.05-27.84 (19.37)
Base of the 2nd dorsal fin	42.17	25.90-53.45 (39.61)
Base of the anal fin	45.66	24.84-56.07 (39.38)
Pectoral fin length	25.37	15.76-31.22 (20.89)
Ventral fin length	16.12	10.39-24.28 (15.28)
D	XI, I + 21	X or XI, I + 20-22
P	16	15-18
V	I + 5	I + 5
A	II + 22	II + 21 - 23
Gill rakers	2 + 8	2-4 + 6-8
Vertebrae	37	37-39
Scales above lateral line	8	7 or 8
As % of BL		
Body depth (BD)	14.11	11.26-18.36 (15.44)
Head length (HL)	27.78	24.71-29.75 (27.00)
Length of caudal peduncle (LCP)	10.65	9.78-14.09 (11.81)
As % of HL		
Upper jaw length (UJL)	21.96	14.08-26.16 (20.90)
Eye diameter (ED)	17.09	15.38-22.66 (18.77)
Interorbital width (IW)	23.13	18.97-28.11 (22.76)
Snout length (SL)	42.19	33.82-45.10 (40.37)
Depth of caudal peduncle (DCP)	21.17	19.26-29.98 (24.86)
LCP/DCP	55.20	48.21-68.38 (57.02)

of anus. Comparisons of meristic characters of 6 *Sillago* species (in our study) and 4 *Sillago* species from Taiwan (Shao et al. 1986, Shen et al. 1993) are shown in table 2.

Color of fresh specimens: Upper surface of head and trunk yellowish-brown, grading to silver on abdomen. Dorsal side of snout brownish-gray. Cheek slightly silver. A faint mid-lateral stripe usually present. Dorsal fin yellowish-hyaline, small dark dense spots on 1st dorsal fin and approximately 3 or 4 rows of clear dusky spots on fin membrane of 2nd dorsal fin. Pectoral, ventral, and anal fins yellowish; caudal fin yellowish-dusky with 2 black margins and grayish-brown margin posteriorly, lobes usually broadly truncate posteriorly.

Habitat: Possibly inhabits tidal flats in estuarine areas, but further studies are needed.

Distribution: *Sillago sinica* sp. nov. is presently only found in coastal waters of the East China Sea, Bohai Sea, and Yellow Sea, China (Fig. 2).

Comparison of otoliths among 6 *Sillago* species

Otoliths are calcareous structures that act as statoliths in the internal ear of fish (Su 2005) and are well known for their time-recording structure that is widely used in studies to assess age (Lowerre-Barbieri et al. 1994, Álvarez et al. 2007). In addition, otolith microscopic examination and measurements are often used to differentiate fish stocks (Tuset et al. 2006). Differences among otoliths in different species reflect varying deposition rates which are mainly affected by metabolic rates and other genetic factors. Therefore, otoliths can be used as aids to identify species. Morphological descriptions of otoliths of 6 *Sillago* species are as follows (Fig. 3).

Sillago bassensis (Fig. 3A): Approximately D-shaped; thicker than those of the other 5 *Sillago* species. The main groove is small, linear, throughout the otolith, with an opening at both ends. No wavy protuberant edges were noted. On the no-groove side, there are some cracks along the middle to the dorsal edge.

Sillago aeolus (Fig. 3B): Pyriform. Most main grooves have rimes throughout the otoliths, which are wider in the center. The no-groove side is smooth, the same as in *S. sihama*, with radial stripes and tumor-like rimes in the middle. The ventral edge is straight, and the dorsal edge is oval; both are smooth and without obvious protuberances.

Sillago japonica (Fig. 3C): Oval, with a visible central core. The main groove is linear, with an opening at both ends. Different from *S. sihama*, there is a crack at the ventral edge. The edge of the undeveloped otolith has wave-like protuberances. The dorsal edge of a developed otolith is smooth, and there are indistinct wavy protuberances on the ventral edge.

Sillago sihama (Fig. 3D): Roughly triangular. Most of the edge is smooth, with only slight protuberances at the posterior end. There is a main groove throughout the otolith, with an opening at both ends. The surface of the no-groove side

Table 2. Comparisons of meristic characters of 6 *Sillago* species (in our study) and 4 *Sillago* species from Taiwan (Shao et al. 1986, Shen et al. 1993)

Species	*S. bassensis*	*S. aeolus*	*S. japonica*	*S. sihama*	*S. parvisquamis*
1st dorsal fin	XI	XI	X or XI	XI	XII or XIII
2nd dorsal fin	I,18	I, 19	I, 21-23	I, 20 or 21	I, 21-23
A	II, 19	II, 18	II, 22-24	II, 22	II, 22 or 23
Vertebrae	34	33 or 34	34 or 35	34	38 or 39
Scales above lateral line	6	7 or 8	3 or 4	5 or 6	7-9

Species	*S. sinica* sp. nov.	*S. chondropus*	*S. ingenuua*	*S. asiatica*	*S. microps*
1st dorsal fin	X or XI	XI or XII	XI	XI	XI
2nd dorsal fin	I, 21 or 22	I, 20-22	I, 17	I, 21	I, 19
A	II, 21-23	II, 22 or 23	II, 17	II, 22	II, 19
Vertebrae	37-39	35	33	34	34
Scales above lateral line	7 or 8	6	5	4 or 5	5

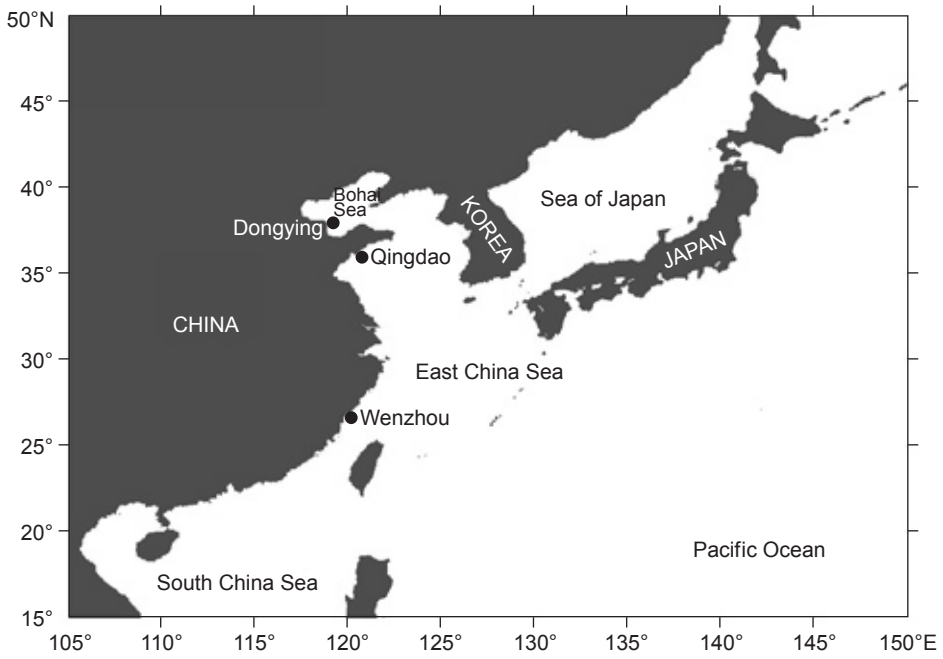

Fig. 2. Sampling sites of 3 *Sillago sinica* sp. nov. populations.

Fig. 3. Otoliths of 6 *Sillago* species. (A) *Sillago bassensis*, (B) *S. aeolus*, (C) *S. japonica*, (D) *S. sihama*, (E) *S. parvisquamis*, (F) *S. sinica* sp. nov.

is bright, with radial stripes and tumor-like rimes in the middle. There is a distinct protuberance on the ventral edge.

Sillago parvisquamis (Fig. 3E): Sunflower seed-shaped. The angle of the anterior portion is significantly smaller than those in the other 5 species. The main groove is S-shaped, with an opening at both ends. There are some indistinct protuberances on the ventral edge, and the dorsal edge side is arced and smooth. The no-groove side has radial rings and verrucous protuberances.

Sillago sinica sp. nov. (Fig. 3F): Kernel-shaped. It obviously differs from those of the other 5 species. The biggest difference is its wavy edge, and there are more wave-like protuberances on the ventral edge than on the dorsal edge. The main groove is linear, near the ventral edge, with only an opening at 1 end. The no-groove side is smooth, and with grape-shaped rimes.

Sequential analysis of the COI gene

Thirty-four specimens of 6 *Sillago* species were used in the analysis. After sequence alignment, a 609-bp fragment of the COI gene was obtained. Ten specimens of *S. sinica* sp. nov. collected at 3 sites yielded identical sequences. There were no indels/insertions, and 204 variable sites were observed in the 34 specimens of 6 *Sillago* species.

Net genetic distances (K2P) within and between species are shown in table 3. Net distances between pairs of the 6 *Sillago* species ranged 0.163-0.220, and values of differentiation between *S. sinica* sp. nov. and *S. parvisquamis*, *S. aeolus*, *S. sihama*, *S. japonica*, and *S. bassensis* were 0.163, 0.188, 0.203, 0.205, and 0.212, respectively.

In total, 202 amino acids coded by 609 bp of the COI gene were used for the differentiation analysis. Three amino acid substitutions were found at the 1st codon and 1 amino acid substitution at the 2nd codon, all of which were caused by non-synonymous substitutions.

The NJ tree was constructed based on the K2P model. Bootstrap values were tested by 1000 replicates. *Sillaginodes punctatus* was initially chosen as the outgroup to root the tree. A parsimony analysis of the COI gene (Fig. 4) revealed that all previously recognized and newly discovered *S. sinica* sp. nov. individuals formed monophyletic groups in the strict consensus trees. The new species *S. sinica* sp. nov. was monophyletic. The phylogenetic tree was mainly divided into 4 clades. *Sillago sinica* sp. nov. and *S. parvisquamis* were clustered in 1 clade, indicating their close relationship. Furthermore, *S. aeolus* was sister to the *S. sinica* sp. nov./*S. parvisquamis* clade, with *S. bassensis* forming a single clade. The *Sillaginodes punctatus* clade was on the outermost part of the tree.

Key to *Sillago* Species of China

1. Body with dark blotches or spots 2
- Body without dark blotches or spots 3
2. Back and upper sides of body with a series of oblique bars usually formed of lines of dark spots, no dark blotch at base of pectoral fin ..... *S. bassensis*
- Body with irregular dark blotches, base of pectoral fin with a dark blotch ..... *S. aeolus*
3. Second dorsal fin membrane usually with spots; bases of anal and ventral fins yellowish; > 4 scales above lateral line 4
- Second dorsal fin membrane mostly hyaline; base of anal and ventral fins hyaline; 3 or 4 scales above lateral line ..... *S. japonica*
4. Second dorsal fin membrane with clear spots; 7-9 scales above lateral line 5
- Second dorsal fin membrane with dusky spots; 5 or 6 scales above lateral line ..... *S. sihama*
5. Scales above lateral line 7-9; 1st dorsal fin spines normally XII or XIII, 2nd dorsal fin with 5 or 6 rows of dusky spots along rays, gradually decreasing in posterior portion ..... *S. parvisquamis*

Table 3. Net genetic distances (K2P) within (on the diagonal) and between (below the diagonal) the 6 *Sillago* species

	S. bassensis	*S. parvisquamis*	*S. japonica*	*S. aeolus*	*S. sihama*	*S. sinica* sp. nov.
S. bassensis	0.006					
S. parvisquamis	0.215	0.000				
S. japonica	0.202	0.220	0.008			
S. aeolus	0.186	0.201	0.216	0.000		
S. sihama	0.206	0.199	0.198	0.178	0.000	
S. sinica sp. nov.	0.212	0.163	0.205	0.188	0.203	0.000

- Scales above lateral line 7 or 8; 1st dorsal fin spines normally X or XI; 2nd dorsal fin with 3 or 4 rows of dusky spots along rays, gradually decreasing in posterior portion ..... *S. sinica* sp. nov.

DISCUSSION

This study proposes a new species of *Sillago*, namely *S. sinica* sp. nov., defined by morphological and DNA barcoding methods.

Sillago sinica sp. nov. is most similar to *S.*

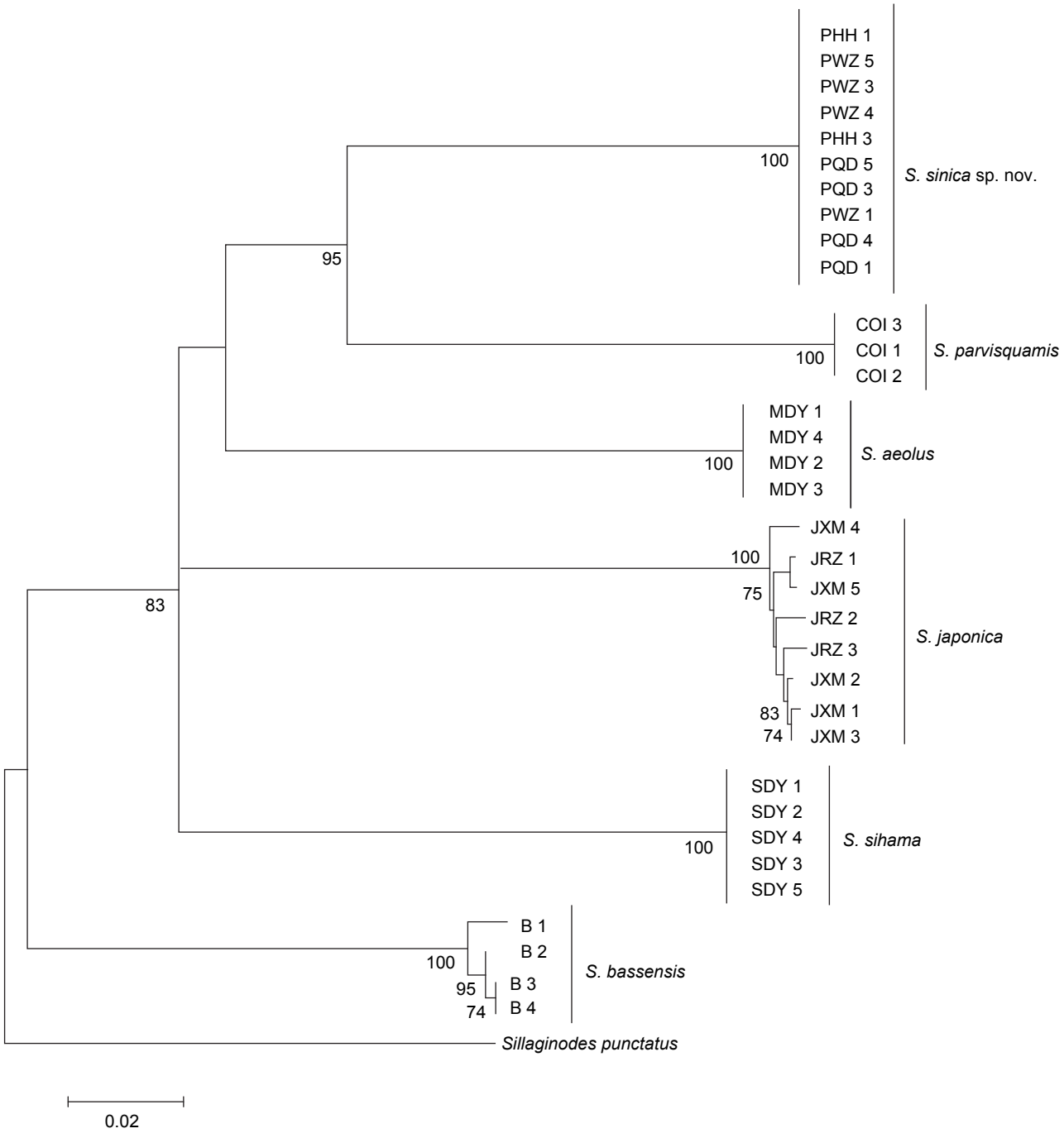

Fig. 4. Neighbor-joining (NJ) tree for cytochrome oxidase subunit I (COI) gene sequences of 7 Sillaginidae species. The NJ tree was constructed under the K2P model using *Sillaginodes punctatus* as the outgroup. Bootstrap support values of > 50% from 1000 replicates are shown.

parvisquamis in general appearance. Both of them have a slender profile and occur in estuarine areas near large rivers. However, the 1st dorsal fin of *S. sinica* sp. nov. is distinguished from the latter by having X or XI spines instead of XII or XIII spines. The new species is further characterized having 3 or 4 rows of dusky spots along the rays of the 2nd dorsal fin instead of 5 or 6 rows of dusky spots in *S. parvisquamis*.

The new species is similar in shape to *S. sihama*, but it is clearly distinguished from the latter by the following features: (1) 5 or 6 scales above lateral line, compared to 7 or 8 scales above lateral line in the new species; (2) caudal fin dusky terminally, whereas the upper and lower edges of the caudal fin are black, and the posterior margin is grayish-brown in the new species; and (3) without a stripe on the body, whereas a faint mid-lateral stripe is present on the new species.

In addition to those 2 species, *S. japonica* is also similar to *S. sinica* sp. nov. in shape and coloration. However, there are 3 or 4 scales above lateral line in *S. japonica*, whereas there are 7 or 8 scales above the lateral line in *S. sinica* sp. nov. Moreover, numbers of vertebrae and scales above the lateral line in *S. asiatica*, *S. chondropus*, *S. ingenuua*, and *S. microps* are both less than those of *S. sinica* sp. nov. (Table 2). Based on these comparisons and the key to the 6 species of *Sillago* given above, identification of the 6 *Sillago* species in our study and 4 species from Taiwan is possible.

Results of the phylogenetic analysis are in agreement with the morphological data. All *Sillago* species were monophyletic at 100% bootstrap values, although *S. sinica* sp. nov. was grouped with *S. parvisquamis*, and *S. japonica* was grouped with *S. sihama* in the COI parsimony analysis. Genetic distances between *S. sinica* sp. nov. and other *Sillago* species based on the K2P model ranged 0.163-0.212, which indicated that the COI gene used as a barcode was effective at identifying *Sillago* species (Ward et al. 2005). The results also indicated the distinctiveness of the new species.

DNA sequence analyses have been used for 30 yr to assist species identifications. Tautz et al. (2003) made the case for a DNA-based taxonomic system. MtDNA analyses have emerged as powerful approaches to resolve questions about fish taxonomy, species identification, and population genetics (Hsu et al. 2009, Lu et al. 2009, Nalugwa et al. 2010, Ng et al. 2010). We can identify species using morphological characters and DNA barcoding. This will play an important role in the study of biodiversity and sustainable development of fishery resources in the future.

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