

A New Record of Sea Urchin (Echinoidea: Camarodonta: Strongylocentrotidae) Based on Morphological and Molecular Analysis in Korea

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ABSTRACT

Some echinoids were collected from the coast of Gangwon-do during the period from November 2008 to July 2011 and were identified on the basis of morphological characteristics and molecular analysis of cytochrome oxidase subunit I mitochondrial DNA. Among them, *Strongylocentrotus pallidus* (Sars, 1871) belonging to the family Strongylocentrotidae of the order Camarodonta is reported for the first time in Korea and is redescribed. The genetic differences ranged from 0.038 to 0.139 between *S. pallidus* and four other species of genus *Strongylocentrotus*, but ranged from 0.002 to 0.005 between Korean specimens and GenBank data of *S. pallidus*. This species is widely distributed in cold sea water along the western part of the North Pacific and the Northwest Atlantic.

Keywords: Echinoidea, sea urchin, *Strongylocentrotus pallidus*, cytochrome oxidase subunit I

INTRODUCTION

The genus *Strongylocentrotus* of the family Strongylocentrotidae consists of nine species globally (Smith, 2005; Kroh and Mooi, 2011): *S. djakonovi*, *S. droebachiensis*, *S. franciscanus*, *S. intermedius*, *S. nudus*, *S. pallidus*, *S. polyacanthus*, *S. pulchellus* and *S. purpuratus*. Among them, *S. intermedius* and *S. nudus* were reported in Korea (Shin and Rho, 1996; Shin, 1998, 2011). The former inhabits only the coast of East Sea but the latter inhabits all coastlines of South Korea except Jeju-do Island (Shin, 2011). Some echinoids were collected from Daejin to Imwon harbors in Gangwon-do and were identified. Among them, *S. pallidus* is newly reported in Korea. Mortensen (1943) reported that morphological characteristics of *S. pallidus* were not distinctly distinguished from the adjacent species such as *S. echinoides* and *S. sachalinicus*. Recently, these two species were recorded as synonymous with *S. pallidus* by morphological and molecular evidences (Jensen, 1974; Tatarenko and Poltarous, 1992; Bazhin, 1998). Therefore, we examined thoroughly morphological characteristics of Korean *Strongylocentrotus* species and analyzed the molecular differences between Korean species and Genbank data of *S. pallidus* and then other

adjacent species using cytochrome oxidase subunit I (COI) mitochondrial DNA (mtDNA).

MATERIALS AND METHODS

Sample collection and identification

The specimens of *Strongylocentrotus* were collected using fishing nets at depths of 50-190 m from nine coastal areas in Gangwon-do from November 2008 to July 2011 (Table 1). Specimens were preserved in 95% methyl alcohol, and their important morphological characters were photographed by light- and stereo-microscopes (Nikon Eclipse 80i, Nikon SMZ1000; Nikon Co., Tokyo, Japan). Identification of specimens referred to Mortensen (1943), Southward and Campbell (2006) and Shin (2011).

DNA amplification and sequencing

Genomic DNA was extracted from the gonad tissues of echinoids using by DNeasy blood and tissues kit (Qiagen, Hilden, Germany) and the COI gene was amplified using primers of Knott and Wray (2000): ECO1a (5'-ACCATGC AACTAAGACGATGA-3') and ECO1b (5'-GGTAGTCTG

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AGTATCGTCGWG-3'). PCR amplification chemistry containing 1.5 μ L of genomic DNA, 2.5 μ L of 10 \times PCR buffer (contained $MgCl_2$), 1.0 μ L of 2.0 mM dNTPs and each primer, 0.3 μ L of nTaq DNA polymerase (Enzynomics, Seoul, Korea) and add up to 25.0 μ L with distilled water, and the following conditions: initial denaturation of 2 min at 95°C, 30 cycles of 95°C 30 sec; 52°C 1 min; and 72°C 1 min and a final elongation of 7 min at 72°C. DNA fragments were sequenced on an ABI 3730XL sequencer (Applied Biosystems Inc., Foster City, CA, USA) using the ABI Prism BigDye Terminator v3.1 (Applied Biosystems Inc.).

Molecular data analysis

The mitochondrial COI gene was mostly sequenced for this study, but the sequences data of four species which are not distributed in Korea obtained from GenBank (Table 2). COI

sequences were checked and aligned using by BioEdit v.7.0 (Hall, 1999) and genetic distances were calculated according to the Kimura 2-parameter model (Kimura, 1980) using by MEGA5 (Tamura et al., 2011). The phylogenetic relationship of the samples was drawn by using the neighbor-joining (NJ), maximum-likelihood (ML) and Bayesian inference (BI). The NJ tree (Saitou and Nei, 1987) was inferred from Kimura 2-parameter genetic distance with bootstrapped 1,000 times using by MEGA5, and the ML analysis with the GTR+G model, determined with jModeltest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008), with 1,000 bootstrap replications using by PhyML v3.0 (Guindon and Gascuel, 2003) also BI analysis with same model and analyzed by MrBayes 3.1 with 1×10^6 generation repeats with the nucleotide model 4by4, Nst=6, rates=gamma, Ngammacat=6, and Burnin= 2.5×10^5 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; Ronquist et al., 2005).

Table 1. Examined materials of Korean echinoids

Scientific name	Date	Location/Depth	No. of specimens
<i>S. pallidus</i>	11 Aug. 2008	Sacheon/100 m	7
	12 Nov. 2008	Jangho/70 m	3
	27 Dec. 2009	Gisamun/50 m	7
	17 Oct. 2010	Daejin/150 m	2
	17 Oct. 2010	Daejin/100 m	8
	18 Oct. 2010	Namae/80 m	12
	24 Dec. 2010	Geojin/120 m	8
	22 Jul. 2011	Daejin/140 m	7
		Gonghyeonjin/120 m	7
	23 Jul. 2011	Jumunjin/190 m	6
	24 Jul. 2011	Imwon/50 m	11
	24 Jul. 2011	Imwon/70 m	2
<i>S. intermedius</i>			
<i>S. nudus</i>	22 Jul. 2011	Gonghyeonjin/120 m	2
<i>H. crassispina</i>	17 Jan. 2011	Munseom/9 m	3

All collection sites locate in Gangwon-do except Jeju Island of *H. crassispina*. *S.*, *Strongylocentrotus*; *H.*, *Heliocidaris*.

RESULTS

Systematic notes

Class Echinoidea Leske, 1778
 Subclass Euechinoidea Bronn, 1860
 Order Camarodonta Jackson, 1912
 Infraorder Echinidea Kroh and Smith, 2010
 Family Strongylocentrotidae Gregory, 1900
 Genus *Strongylocentrotus* Brandt, 1835

¹*Strongylocentrotus pallidus* (Sars, 1871)

Toxopneustes pallidus Sars, 1871: 25.

Strongylocentrotus pallidus Bidekap, 1899: 112; Jensen, 1974: 119; Vader et al., 1986: 10; Southward and Campbell, 2006: 144; Kroh and Mooi, 2011: 124324.

Table 2. List of taxa included in this study, higher taxonomic placement and GenBank accession numbers for their gene sequences

Family	Scientific name	COI
Echinometridae	<i>Heliocidaris crassispina</i> (A. Agassiz, 1863)	JN716400
Strongylocentrotidae	<i>Allocentrotus fragilis</i> (Jackson, 1912) ^a	HM473956
	<i>Strongylocentrotus droebachiensis</i> (Müller, 1776) ^a	HM542394
	<i>Strongylocentrotus intermedius</i> (A. Agassiz, 1863)	JN716399
	<i>Strongylocentrotus nudus</i> (A. Agassiz, 1863)	JN716398
	<i>Strongylocentrotus pallidus</i> (Sars, 1871) (A)	JN716393
	<i>Strongylocentrotus pallidus</i> (Sars, 1871) (B)	JN716394
	<i>Strongylocentrotus pallidus</i> (Sars, 1871) (C)	JN716395
	<i>Strongylocentrotus pallidus</i> (Sars, 1871) (D)	JN716396
	<i>Strongylocentrotus pallidus</i> (Sars, 1871) (E)	JN716397
	<i>Strongylocentrotus pallidus</i> (Sars, 1871) ^a	HM543071
	<i>Strongylocentrotus polyacanthus</i> A. Agassiz and H.L. Clark, 1907 ^a	EU700091

Strongylocentrotus pallidus (A)-(E) were indicated in Table 3. COI, cytochrome oxidase subunit I. ^aObtained from GenBank.

Korean name: ¹*연약둥근성게 (신칭)

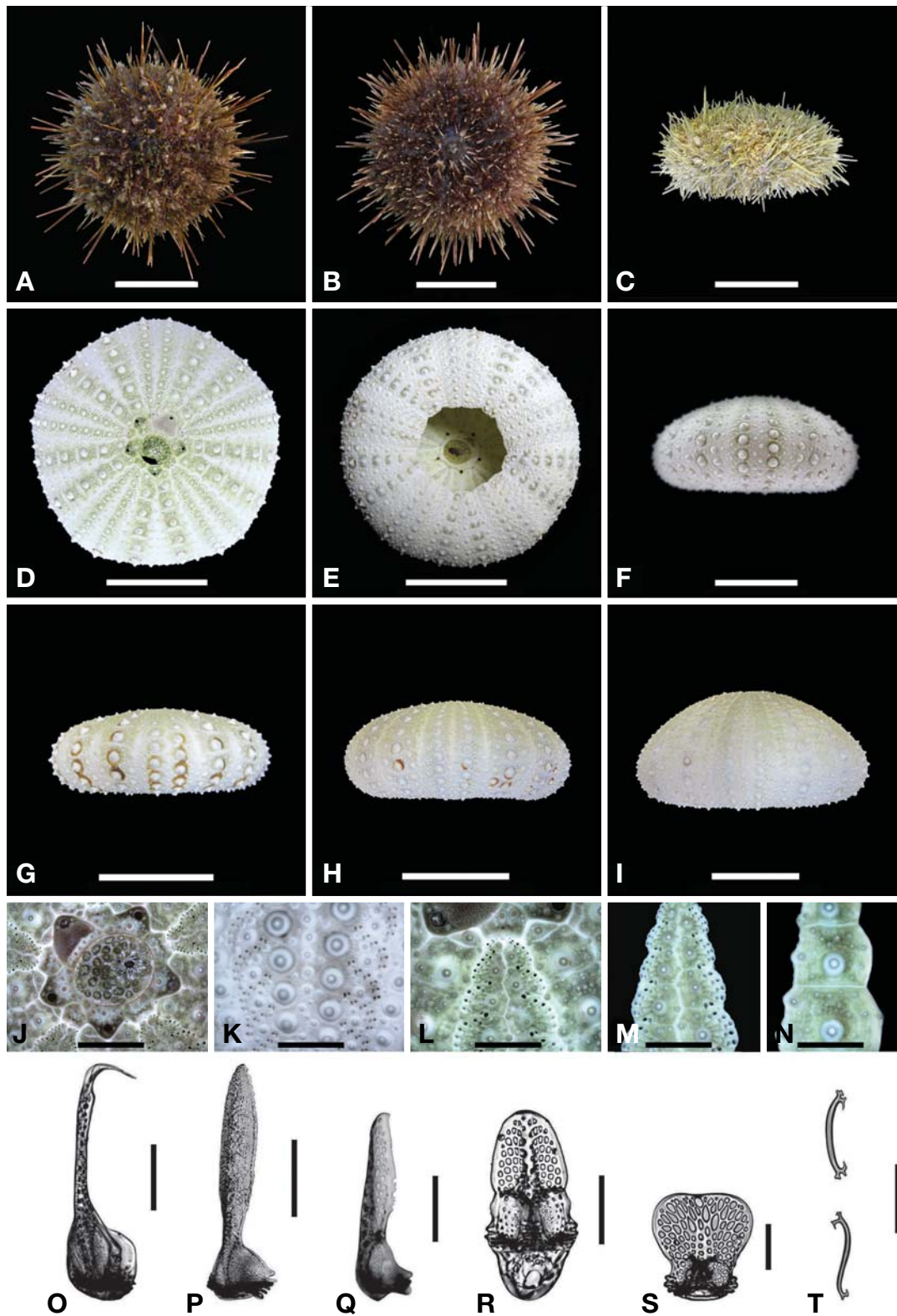


Fig. 1. *Strongylocentrotus pallidus*. A, Dorsal side; B, Ventral side; C, Lateral side; D, Dorsal side of denuded test; E, Ventral side of denuded test; F-I, Lateral side of denuded test; J, Apical system; K-M, Ambulacral plates; N, Interambulacral plates; O, Globiferous pedicellaria; P, Q, Large and small tridentate pedicellaria; R, Ophiocéphalous pedicellaria; S, Triphyllous pedicellaria; T, Spicules of tube-feet. Scale Bars: A-I=2.5 cm, J-N=0.5 cm, O, P=300 nm, Q, R=200 nm, S, T=100 nm.

Table 3. Morphological characteristics of species of *Strongylocentrotus* in Korea

	Size of test					No. of pore-pairs	No. of plates		Shape of test	
	D (mm)	H (mm)	P (mm)	H/D (%)	P/D (%)		Ambulacral plate	Interambulacral plate	Horizontal	Vertical
<i>S. intermedius</i>	28.0-60.0	14.0-35.0	11.0-19.0	47.0-51.0	32.0-39.0	5	25-40	19-29	Circular	Hemispherical
<i>S. nudus</i>	20.0-100.0	11.0-53.0	9.0-32.0	45.0-50.0	36.0-41.0	6	13-30	11-19	Circular	Hemispherical
<i>S. pallidus</i>	43.0-59.0	16.5-29.5	14.5-19.7	38.2-50.0	31.6-35.7	6	20-24	14-16	Roundly pentagonal	Flattened-hemispherical
(A)	44.0	16.5	14.5	38.2	33.0	6	20	14	"	Flattened
(B)	46.0	20.0	16.3	43.4	35.4	6	20	14	"	Low hemispherical
(C)	55.0	23.7	18.3	43.1	33.3	6	24	16	"	"
(D)	57.0	25.5	18.0	43.9	31.6	6	24	16	"	"
(E)	56.0	28.0	19.7	49.1	35.2	6	24	16	"	Hemispherical

(A)-(E) of *S. pallidus* used for the molecular analysis. Morphological data of *S. intermedius* and *S. nudus* are referred from Shin (2011). D, diameter; H, height; P, peristome.

Strongylocentrotus droebachiensis var. *sachalinicus* Döderlein, 1906: 517.

Strongylocentrotus echinoides A Agassiz and HL Clark, 1907: 122; HL Clark, 1912: 360; Mortensen, 1943: 219; Downey, 1968: 82.

Strongylocentrotus sachalinicus HL Clark, 1912: 353; Mortensen, 1943: 215; Kroh and Mooi, 2011: 513826.

Strongylocentrotus droebachiensis sachalinica D'yakonov, 1938: 470, 496.

Key to the species of genus *Strongylocentrotus* in Korea

1. Ambulacral pore-pairs five in number *S. intermedius*
 Ambulacral pore-pairs six in number 2
2. Primary spines long and stout *S. nudus*
 Primary spines short and not stout *S. pallidus*

Description. Test form vertically very variable (Table 3); flattened (Fig. 1G), low-hemispherical (Fig. 1F, H) and hemispherical forms (Fig. 1I). Outline of test roundly pentagonal forms (Fig. 1D). Margin of oral side slightly sunken towards peristome. Test of largest specimen 59 mm in diameter. Six ambulacral pore-pairs presented in an erected arc (Fig. 1K-M). Madreporite has convexed form, larger than genital plates (Fig. 1J). Ocular plates composed five plates, three plates has pentagonal form, stuck between genital plates, like wedges, the other two plates have hexagonal form, situated between genital plates, bordering surnal parts. Surnal parts consist of numerous small plates, with anus situated beside center, surrounded with small blunt spines (Fig. 1J). Globiferous pedicellaria has single slender apical tooth, without lateral tooth (Fig. 1O). Tridentate pedicellaria has two different sizes, large and small form; large ones about five times larger than small one (Fig. 1P, Q). Ophiocephalous pedicellaria with fountain pore-pattern valves (Fig. 1R).

Triphyllous pedicellaria apple-like shaped, with upraised radial pore-pattern (Fig. 1S). Spicule of tube-foot usually elongated arch form, rarely twisted form, which has trifurcated end, of which inside node slender, tapered to tip (Fig. 1T).

Distribution. Korea (Gangwon-do), Japan (Siaukhu Bay), Bering Sea, Okhotsk Sea, North Pacific (Kuril island, Sakhalin), Northwest Atlantic (Norway, U.K.).

Molecular analysis

DNA sequence features. A total of 1,214 base pairs (bp) of the COI mtDNA were obtained from five specimens of *Strongylocentrotus pallidus* and the other Korean echinoids such as *S. intermedius*, *S. nudus* and *Heliocidaris crassispina* (Table 2). But, GenBank sequences were shorter than our sequences and so 818 bp COI mtDNA were analyzed in this study. In a group of *S. pallidus*, only 4 bp of the 818 bp was different from each.

Phylogenetic tree. The phylogenetic relationships of the COI mtDNA from *Strongylocentrotus* species were analyzed by BI, ML and NJ method. We appointed *Heliocidaris crassispina* as the outgroup, and analyzed with *S. droebachiensis*, *S. pallidus*, *S. polyacanthus* and *Alloccentreotus fragilis* obtained from GenBank. In the phylogenetic trees, BI, ML and NJ analyses represented the non-discriminatory phylogenetic branches (Figs. 2, 3). Korean specimens of *S. pallidus* coincident with *S. pallidus* of GenBank, and our phylogenetic reconstruction suggests that the genus *Strongylocentrotus* with *A. fragilis* is paraphyletic, but *A. fragilis* is closely related to *S. pallidus* and *S. droebachiensis* and its taxonomical position still obscure. Therefore the genus *Strongylocentrotus* without *A. fragilis* is monophyletic.

Genetic distances. Kimura 2-parameter genetic distance between *Strongylocentrotus pallidus* (Korea) and *S. pallidus*

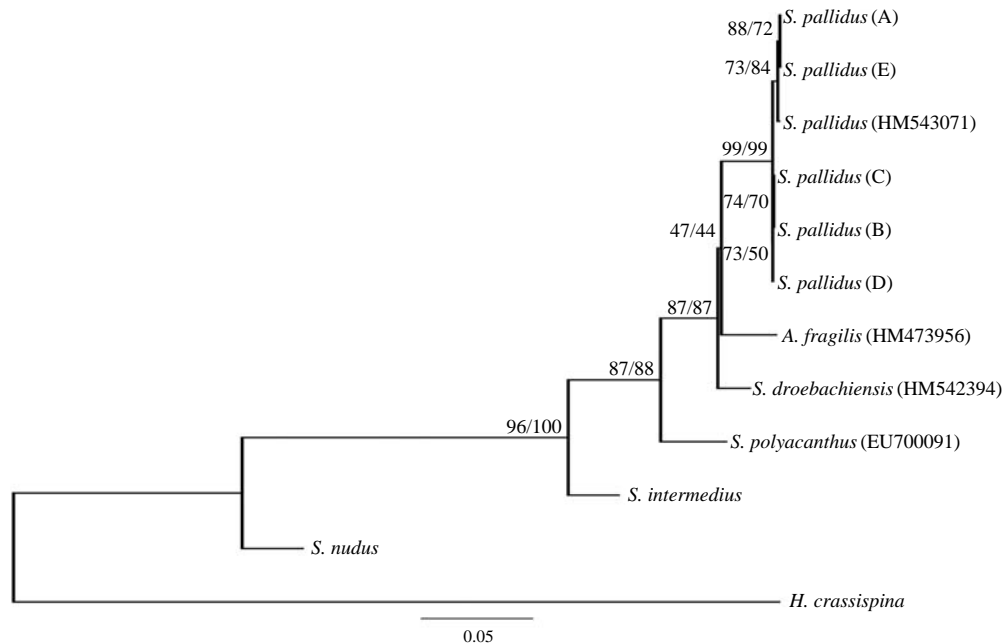


Fig. 2. Maximum likelihood (ML) and neighbor joining (NJ) combined tree generated from the COI mtDNA dataset. Node values following ML/NJ. *S.*, *Strongylocentrotus*; *A.*, *Allocentrotus*; *H.*, *Heliocidaris*; COI, cytochrome oxidase subunit I; mtDNA, mitochondrial DNA.

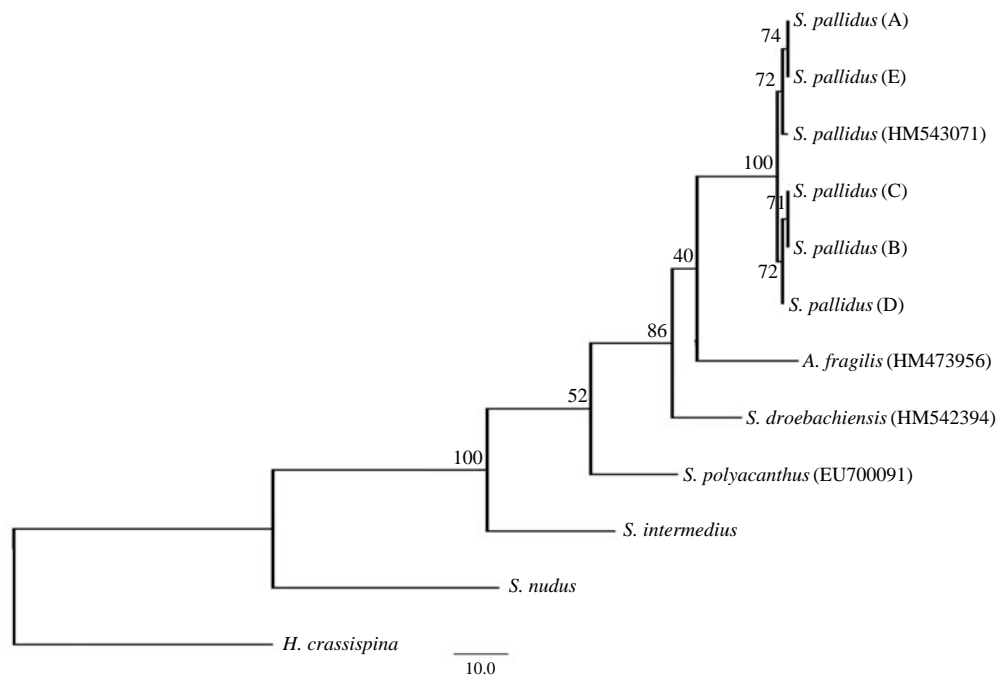


Fig. 3. Bayesian inference (BI) tree generated from the COI mtDNA dataset. *S.*, *Strongylocentrotus*; *A.*, *Allocentrotus*; *H.*, *Heliocidaris*; COI, cytochrome oxidase subunit I; mtDNA, mitochondrial DNA.

(GenBank) ranged from 0.002 to 0.005 (Table 4). Pairwise (*p*) distance average of the *Strongylocentrotus* group is 0.062 and excepted *S. nudus* of *Strongylocentrotus* group is 0.041.

Average of between *S. pallidus* and *Allocentrotus* is 0.045, which was lower than average of the *Strongylocentrotus* group, and between *S. pallidus* and *S. droebachiensis* dis-

Table 4. Interspecific pairwise (*p*) distance values among six species of genus *Strongylocentrotus* with an outgroup (*Heliocidaris crassispina*) based on partial sequences of mtDNA COI gene, which determined by the Kimura 2-parameter model

Species	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>S. pallidus</i> (A)												
2. <i>S. pallidus</i> (B)	0.005											
3. <i>S. pallidus</i> (C)	0.005	0.001										
4. <i>S. pallidus</i> (D)	0.004	0.001	0.001									
5. <i>S. pallidus</i> (E)	0.000	0.004	0.005	0.005								
6. <i>S. pallidus</i> (HM543071) ^a	0.002	0.004	0.005	0.005	0.002							
7. <i>S. droebachiensis</i> (HM542394) ^a	0.039	0.038	0.039	0.039	0.039	0.038						
8. <i>S. intermedius</i>	0.081	0.080	0.081	0.081	0.081	0.081	0.083					
9. <i>S. nudus</i>	0.139	0.134	0.136	0.136	0.139	0.136	0.127	0.124				
10. <i>S. polyacanthus</i> (EU700091) ^a	0.065	0.063	0.065	0.065	0.065	0.065	0.056	0.070	0.125			
11. <i>A. fragilis</i> (HM473956) ^a	0.046	0.045	0.043	0.043	0.046	0.046	0.044	0.084	0.137	0.066		
12. <i>H. crassispina</i>	0.214	0.216	0.214	0.214	0.214	0.216	0.213	0.205	0.188	0.215	0.211	

S., *Strongylocentrotus*; *A.*, *Alloccentrotus*; COI, cytochrome oxidase subunit I; mtDNA, mitochondrial DNA. ^aObtained from GenBank.

tance (=0.039) was lower than the *Strongylocentrotus* group average.

DISCUSSION

Two species of *Strongylocentrotus* (*S. intermedius* and *S. nudus*) were previously reported (Shin, 2011). In this study, *S. pallidus* is newly reported, which has distinct characteristics compared with two species: external figure, pedicellariae and number of pore-pairs in an arc. *Strongylocentrotus pallidus* has various range of test height; the range of height/diameter of Korean specimens is 38.2-50.0% and range of peristome/diameter is 31.6-35.7% (Table 3). However, Mortensen (1943) described the range of peristome/diameter as 31.6-45.0%. Thus, we divided the three groups based on the test height: Group-1 (flattened form)=*S. pallidus* (A), Group-2 (low-hemispherical form)=*S. pallidus* (B)-(D) and Group-3 (hemispherical form)=*S. pallidus* (E). Korean specimens of *S. pallidus* revealed as identical with *S. pallidus* of GenBank. According to the results of phylogenetic analysis and genetic distance (Figs. 2, 3, Table 4). In the phylogenetic tree, *S. pallidus* group divided by two clades (Figs. 2, 3); *S. pallidus* (A), (E) and *S. pallidus* (B)-(D). But, that is not a significant cladogram, because the intraspecific *p*-distance average value is only 0.003 and is much lower than other interspecific *p*-distances (Table 4).

Strongylocentrotus pallidus is very similar with *S. droebachiensis* and can only be discriminated by rather impalpable differences (Vasseur, 1951; Swan, 1962; Jensen, 1974; Vader et al., 1986). For that reason, it has been included in *S. droebachiensis* (Mortensen, 1943). In these phylogenetic results, *S. pallidus* and *S. droebachiensis* showed rather close relationships (Table 4); *p*-distance value is only 0.039,

which is lower than average of *Strongylocentrotus* (0.062) and in phylogenetic tree, *S. pallidus* were branched off close to *S. droebachiensis*. Thus, echinoid specimens are unrecorded species examined in Korea by determining on the basis of the morphological and molecular evidences. Recently, they were divided from each other on the basis of morphological characters within populations and between geographical districts, genetic differences and capacity for hybridization (Vasseur, 1951; Jensen, 1974; Vader et al., 1986; Biermann et al., 2003).

Mortensen (1943) reported that *Alloccentrotus fragilis* is very unlike any of the true species of *Strongylocentrotus*. However, previous studies (Strathmann, 1979; Biermann et al., 2003) and our phylogenetic results (Figs. 2, 3) show rather close relationships between *A. fragilis* and the species of *Strongylocentrotus*. The taxonomical position of *A. fragilis* needs to be consider in a further study.

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