Korean J. Malacol. 32(3): 221-229 2016
http://dx.doi.org/10.9710/kjm.2016.32.3.221

First record of *Octopus longispadiceus* (Cephalopoda: Octopodidae) from Korea

Jong Bin Kim, Jae-Hyeong Yang and Soo Jeong Lee

East Sea Fisheries Research Institute, National Institute of Fisheries Science, Gangneung, 25435, Korea

ABSTRACT

Fifty-five specimens of *Octopus longispadiceus*, belonging to the family Octopodidae, were collected for the first time from the East Sea of Korea and identified by DNA barcoding. This species is characterized by its long right third arm and ligula, the presence of enlarged suckers in the mature male, small white spots on the mantle, head, and arms, and no cirrus above the eye. A molecular analysis of the partial cytochrome c oxidase subunit I gene showed that these specimens are all the same species and have the smallest genetic distance with *O. longispadiceus* (Kimura- two-parameter distance = 0.002-0.003). A new Korean name, “Bal-mun-eo” is proposed for this species.

Key words: First record, *Octopus longispadiceus*, Octopodidae, Korea

Introduction

The Octopodidae, of the class Cephalopoda, consists of more than 350 species worldwide, although many of these are known only from their original reports (Norman and Hochberg, 2005). Members of the family, which is characterized by having muscular body with eight arms, 1 or 2 rows of suckers, hectocotylized arm (typically right third arm), no fins, and absence of rows of cirri adjacent to suckers, inhabit from intertidal reefs to great depths in all oceans of the world from the equator to polar regions (Jereb *et al*., 2016). Among the Octopodidae, *Octopus* is a cosmopolitan genus and consists of about 90 species worldwide (Nesis, 1987; Kaneko *et al*., 2011). This genus is characterized by muscular arms of 3 to 5 times mantle length, long lateral arms, the deepest webs on lateral arms, 2 rows of suckers, presence of enlarged sucker in mature male and ink sac, and skin with distinct patch and groove. There are five species in Korea: *Octopus minor* (Sasaki, 1920), *O. ocellatus* (Gray, 1849), *O. vulgaris* (Cuvier, 1797), *O. dofleini* (Wülker, 1910), and *O. megalops* (Taki, 1964) (Kim and Chun, 2010).

During a survey of the cephalopod fauna in the East Sea, fifty-five specimens (27 male and 28 female) belonging to the genus *Octopus* were collected. The description of the specimens collected and its identification as *O. longispadiceus* are in good agreement with previous descriptions of the species (Gleadall, 1933; Takeda, 2003). This species has not been reported previously from Korea. In this study, we described its morphological characteristics and provide a new Korean name for the species *Octopus longispadiceus*.

Materials and Methods

1. Sampling

Fifty-five specimens, belonging to genus *Octopus*, were collected from the East Sea by trap or bottom trawl (Fig. 1). The specimens were stored in a freezer and then in 10% formalin. Small tissue samples were taken for molecular analysis from the mantles of the specimens before fixation. All the specimens and tissue samples were deposited in the East Sea Fisheries

Received: September 19, 2016; Revised: September 24, 2016; Accepted: September 30, 2016
Corresponding author : Soo Jeong Lee
Tel: +82 (33) 660-8524 ,e-mail: mercione@korea.kr
1225-3480/24631
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License with permits unrestricted non-commercial use, distribution, and reproducibility in any medium, provided the original work is properly cited.

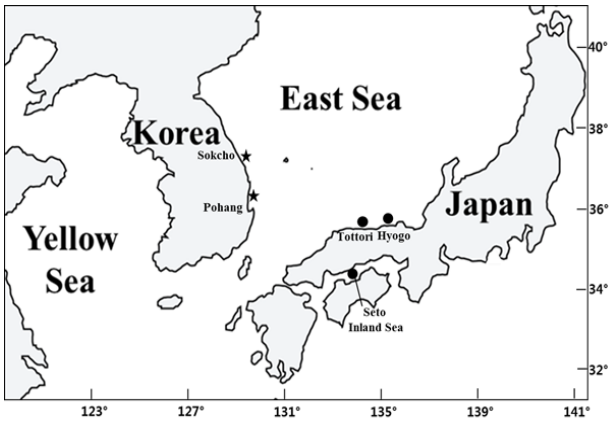

Fig. 1. Map showing the sampling area for *Octopus longispadiceus*. Stars indicate the sampling area in the present study. Circles indicate the sampling areas of the *O. longispadicenus* specimens compared in the morphological and molecular analyses (Hyogo: Takeda 2003; Seto In land Sea: Kaneko *et al*. 2011; Tottori: accession number AB121296 in NCBI).

Research Institute (ESFRI) of the National Institute of Fisheries Science of Korea.

2. Morphological analysis

Measurements were made according to Jereb *et al*. (2016) and the taxonomic system and terminology used follows Norman and Hochberg (2005) and Jereb *et al*. (2016), respectively. Body measurements were made with tape, digital calipers, and plate. Internal organs were investigated by dissection.

3. Molecular analysis

Total DNA was extracted from the muscle tissues with a DNA extraction kit (DNeasy® Blood & Tissue Kit, Qiagen, Netherlands), according to the manufacturer's protocol. The cytochrome c oxidase subunit 1 gene (*COI*) of the mitochondrial DNA (mtDNA) was amplified from each DNA sample with polymerase chain reaction (PCR) and the primer set reported previously by Folmer *et al*. (1994). We also obtained the mtDNA *COI* sequences for the family Octopodidae from the National Center for Biological Information (NCBI) and BOLDSYSTEMS (http://www.boldsystems.org). The sequences were aligned with BioEdit ver. 7 (Hall, 1999) and the genetic distances (*d*) were calculated with the Kimura-two-parameter model (Kimura, 1980). A neighbor-joining tree was constructed with 1,000 bootstrap replications using the MEGA 5 software (Tamura *et al*., 2011). The nucleotide sequence data reported here have been submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases the accession numbers KX758504-KX758514 and, KX758516-KX758531.

Results and Discussion

1. *Octopus longispadiceus* Sasaki, 1917

(Korean name: Bal-mun-eo) (Figs 2-5, Tables 1-2)

Polypus longispadiceus Sasaki, 1917: 366 (type locality: Rikuzen Province, Japan)

Octopus longispadiceus: Robson, 1929: 112, Gleadall, 1993: 145, Takeda, 2003: 29, Kaneko *et al*., 2011: 97.

2. Material examined

Octopus longispadiceus: ESFRI-Octo 1-11 (11), 364-571 mm total length (TL), Pohang-si, Gyeongsangbuk-do, Korea, 100-150 m depth, caught by trap, 22 February., 2016; ESFRI-Octo 12-36 (25), 386-653 mm TL, Pohang-si, Gyeongsangbuk-do, Korea, 100-150 m depth, caught by trap, 24 June, 2016; ESFRI-Octo 37-55 (19), 362-579 mm TL, Sokcho-si, Gangwon-do, Korea, 160-170 m depth, caught by bottom trawl, 19 July, 2016.

3. Description

All measurements are listed in Tables 1 and 2. Body is soft and smooth without warts. No cirrus above the eye. Numerous small white spots on mantle, head, and arms. Mantle length (ML) is 16.4%-30.1% of TL. Ventral mantle length nearly equals mantle width. Mantle opening extends about half the body circumference. Eye is small, length 4.6%-14.1% of ML. Arms are slender and elongate, with the formula I > II > III > IV (42%) or II > I > III > IV (12%); and the longest arm is 63%-81% of TL. The right third arm is hectocotylized and its length is 64%-81% (mean 74%) of the longest arm. Ligula is long and thin, its length is 4%-12% (mean 8%) of the right third arm. Suckers are biserial and suckers 12-15 from the base of the arm are enlarged in the mature male. The gill has

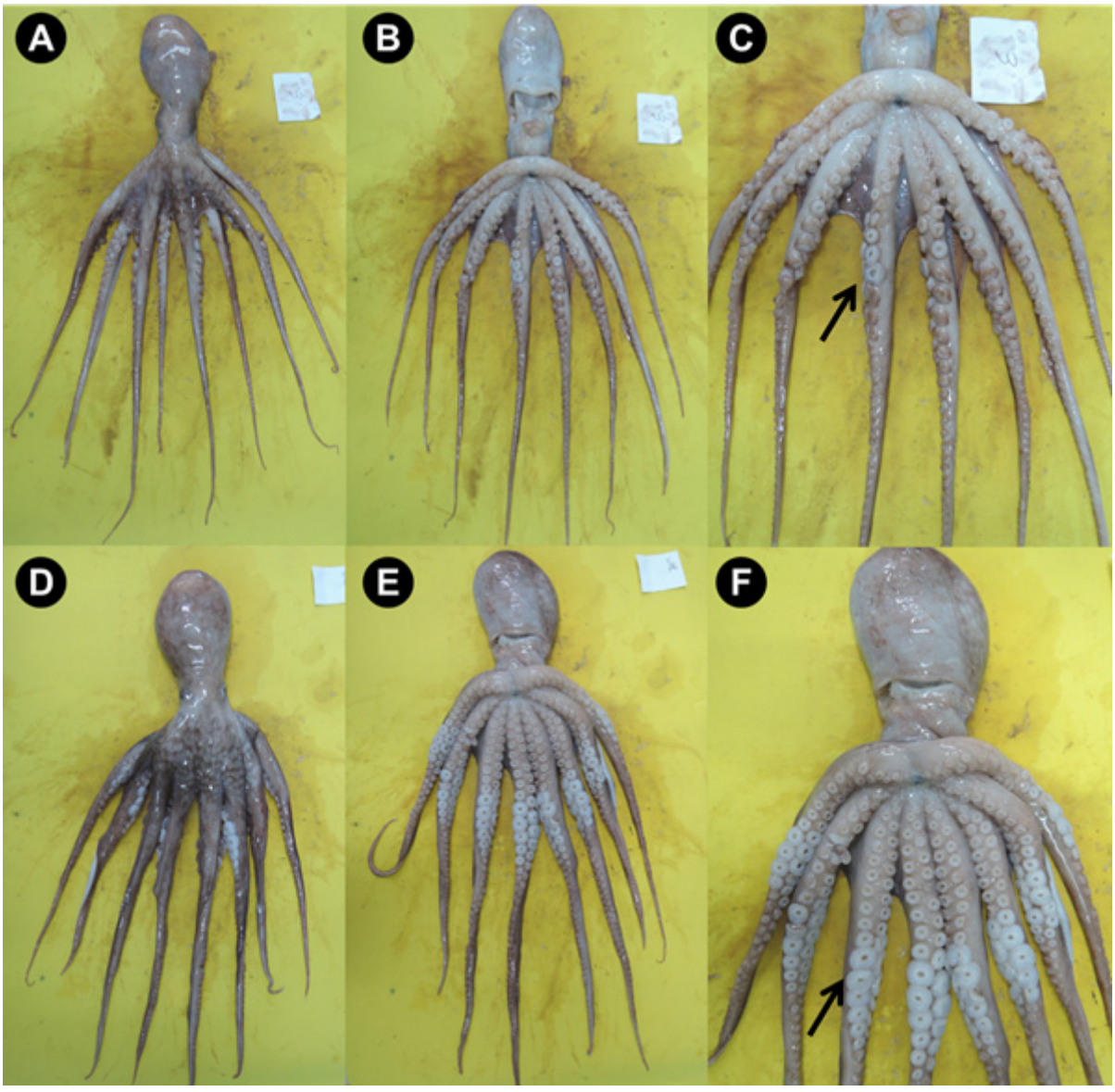

Fig. 2. Male specimens of *Octopus longispadiceus* examined in the present study (frozen). **A-C**: ESFRI-Octo39, 429 mm TL, **D-F**: ESFRI-Octo37, 477 mm TL (A and D: dorsal, B and E: ventral). Arrows in C and F indicate enlarged suckers.

20-22 lamellae. The terminal organ is nearly straight or a little curved in the raw and frozen conditions. Eggs are elliptical and yellowish. Each ovary has 60-120 eggs in the mature female and the major axis of the egg is 10-24 mm.

4. Distribution

Known from the Miya Prefecture, the western coast of Hyogo Prefecture, and Seto Inland Sea in Japan (Gleadall, 1993; Kaneko *et al*., 2011; Takeda, 2003), and the East Sea of Korea (present study)

Remarks

The present specimens were identified as belonging to genus *Octopus* by its muscular arms of about 3 times mantle length, long lateral arms, the deepest webs on lateral arms, 2 rows of suckers, and presence

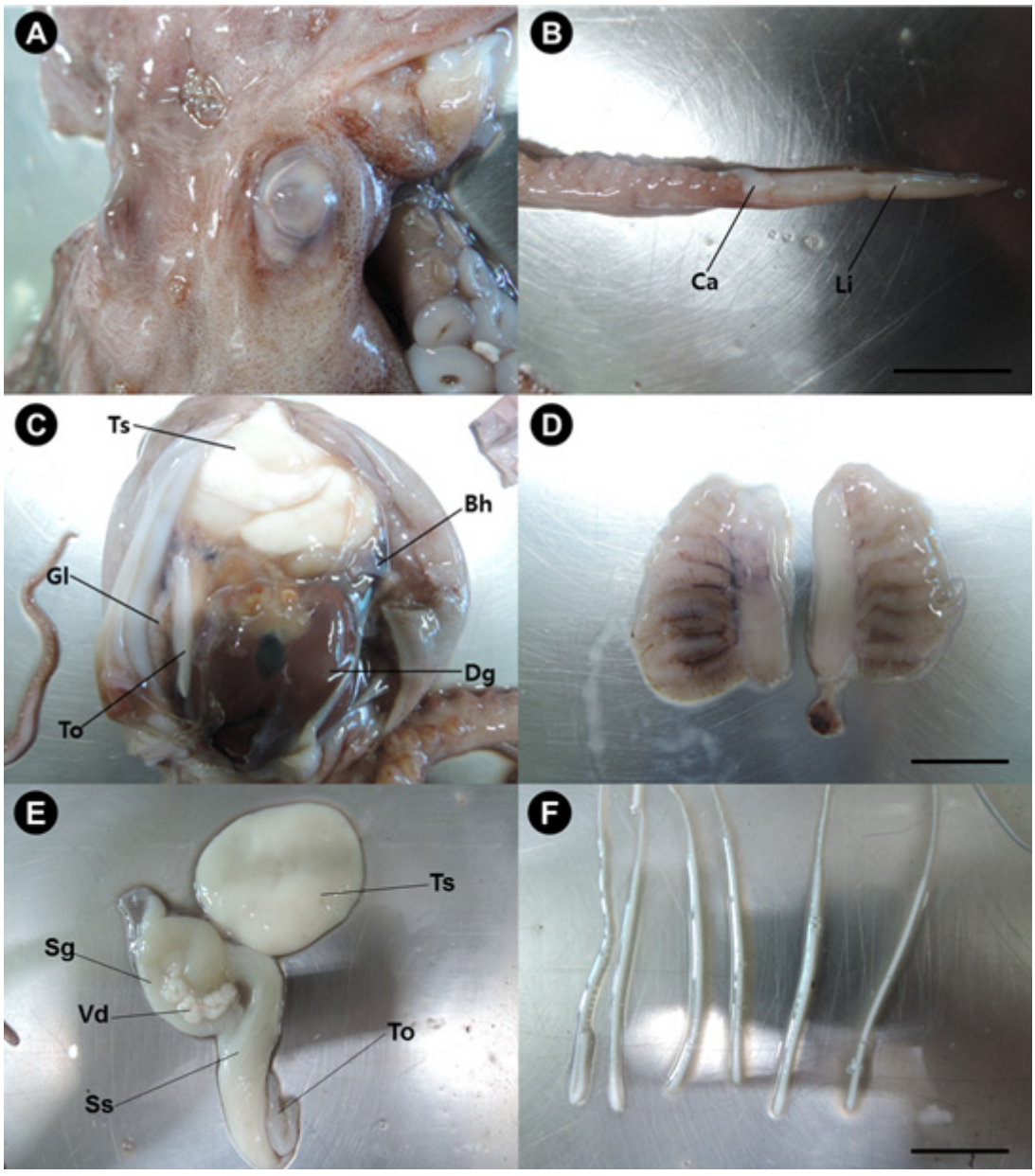

Fig. 3. Male specimen of *Octopus longispadiceus* (ESFRI-Octo39, 429mm TL). **A**: eye, no cirri found above the eye. **B**: end of right third arm (hectocotylus). **C**: dissected mantle. **D**: gill lamella. **E**: male reproductive tract. **F**: Spermatophores. Abbreviations: Bh, branchial heart; Ca, calamus; Dg, digestive gland; Gl, gill lamella; Li, ligula; Sg, spermatophoric gland; Ss, spermatophoric sac; To, terminal organ; Ts, testis; Vd, vas deferens. Bars indicate 1cm. Fig. 4. Female specimen of *Octopus longispadiceus* in the present study (ESFRI-Octo49, 485 mm TL) (frozen). A: Dorsal; B: ventral; C: female reproductive tract; D: eggs. Bar (D) indicates 1 cm.

of enlarged sucker in mature male and ink sac. These specimens were identified as *O. longispadiceus* by its long hectocotylized arm and ligula, white small white spots on the mantle, head, and arms, and dominant arm formula (I > II > III > IV).

Takeda (2003) investigated 506 specimens of *O. longispadiceus* collected from Tajima in Hyogo Prefecture (western coast of Japan) and reported morphological traits similar to our results: dorsal mantle length 25% of TL in *O. longispadiceus* vs 24.5%

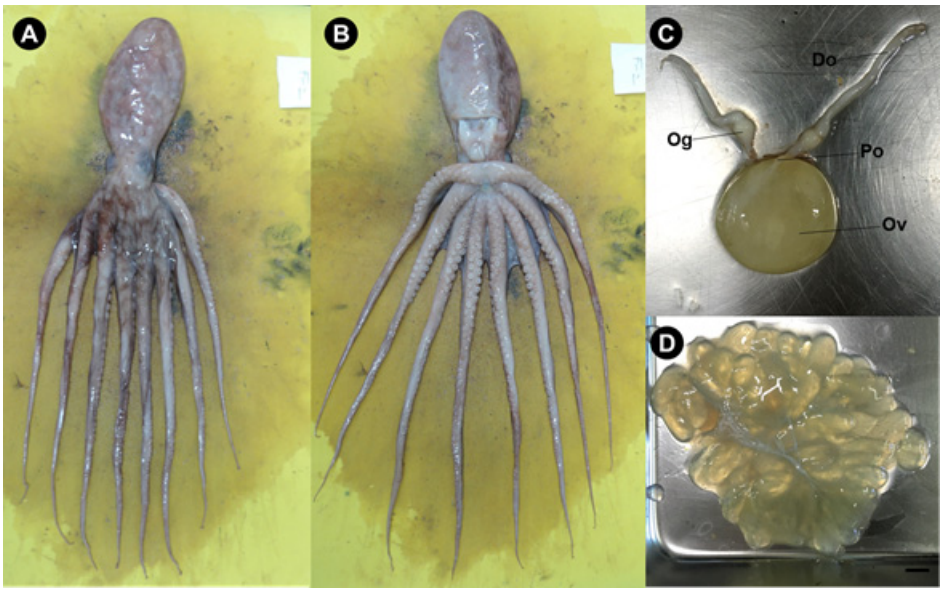

Fig. 4. Female specimen of *Octopus longispadiceus* in the present study (ESFRI-Octo49, 485mm TL) (frozen). **A**: Dorsal, **B**: ventral, **C**: female reproductive tract, **D**: eggs. Abbreviations: Do, distal oviduct; Og, oviducal gland; Ov, Ovary; Po, proximal oviduct. Bar (D) indicates 1cm.

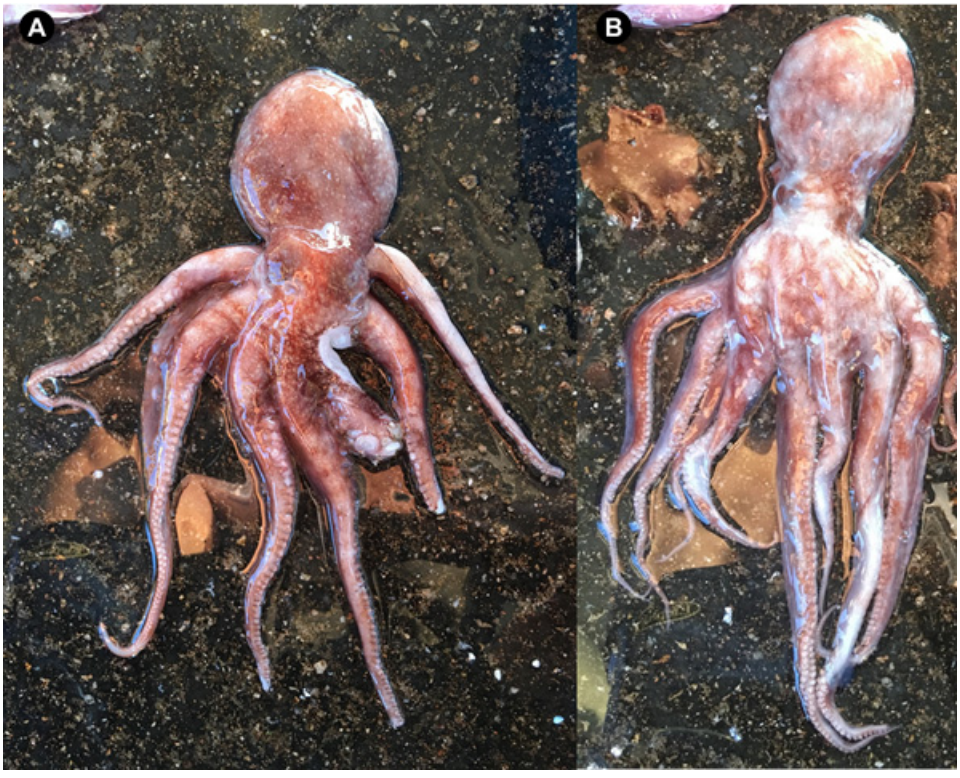

Fig. 5. *Octopus longispadiceus* in the raw condition (photograph taken by author on 12 August, 2016, Yangyang-gun, Gangwon-do, Korea). **A**: Male (about 270 mm TL); **B**: Female (about 350 mm TL). In the raw condition, they have light brown and reddish skin, and small white spots are distributed on the mantle, head, and arms.

Table 1. Comparison of external measurements of *Octopus longispadiceus*

	Present study		Takeda (2003)	Sasaki (1929)
SEX	male	female	male	male
Total length (mm)	362-624	364-653	205-458	265-330
	(n = 27)	(n = 28)	(n = 32)	(n = 3-4)
% of total length				
Dorsal mantle length	23-30 (27)*	17-28 (24)	19-28 (24)	17-22 (20)
Ventral mantle length	13-20 (17)	13-22 (17)	14-22 (19)	14-19 (16)
Mantle width	11-20 (15)	12-19 (15)	13-19 (16)	14-17 (15)
Head width	6-11 (8)	6-10 (8)	8-15 (11)	10-13 (11)
Head length	4-7 (6)	4-7 (5)	-	-
Eye diameter	1.5-3.5 (2.2)	1.1-2.9 (1.9)	-	-
Arm length Right I	57-77 (71)	50-84 (71)	72-83 (76)¥	-
Arm length Right II	50-77 (69)	51-79 (68)	63-83 (70)¥	74-75 (74)
Arm length Right III	45-59 (55)	59-73 (63)	56-75 (66)¥	65-75 (69)
Arm length Right IV	43-71 (59)	50-68 (60)	51-64 (57)¥	62-70 (65)
Arm length Left I	62-77 (70)	44-81 (69)	68-81 (75)¥	74-78 (76)
Arm length Left II	51-77 (67)	51-78 (67)	62-75 (69)¥	69-74 (71)
Arm length Left III	48-72 (61)	46-70 (61)	53-72 (63)¥	62-28 (65)
Arm length Left IV	48-70 (58)	49-68 (59)	50-68 (59)¥	62-69 (66)
Ligula length	2-7 (4)	-	-	-

*11 specimens were measured. ¥9 specimens were measured

Table 2. Comparison of actual measurements of *Octopus longispadiceus* with those of Takeda (2003)

	present study				Takeda (2003)			
Specimens	ESFRI-Octo19	ESFRI-Octo26	ESFRI-Octo4	ESFRI-Octo49	1	2	3	4
Sex	Male	Male	Female	Female	Male	Male	Female	Female
TL (mm)	413	455	453	485	338	458	475	485
DML (mm)	-	-	93	137	73	87	103	92
VML (mm)	84	66	60	94	62	64	90	70
MW (mm)	68	65	55	70	58	59	59	69
HW (mm)	36	33	35	38	35	46	37	48
AL L I (mm)	376	286	366	328	270	321	375	335
AL L II (mm)	348	324	330	338	250	309	337	340
AL L III (mm)	342	278	302	306	215	240	295	325
AL L IV (mm)	256	274	276	274	210	229	280	280
AL R I (mm)	356	312	288	312	270	344	343	370
AL R II (mm)	348	352	332	326	245	286	320	405
AL R III (mm)	306	262	276	316	210	255	311	340
AL R IV (mm)	306	262	274	300	195	235	311	290

TO, total length; DML, dorsal mantle length; VML, ventral mantle length; MW, mantle width; HW, head width; AL, arm length; R, right side; L, left side.

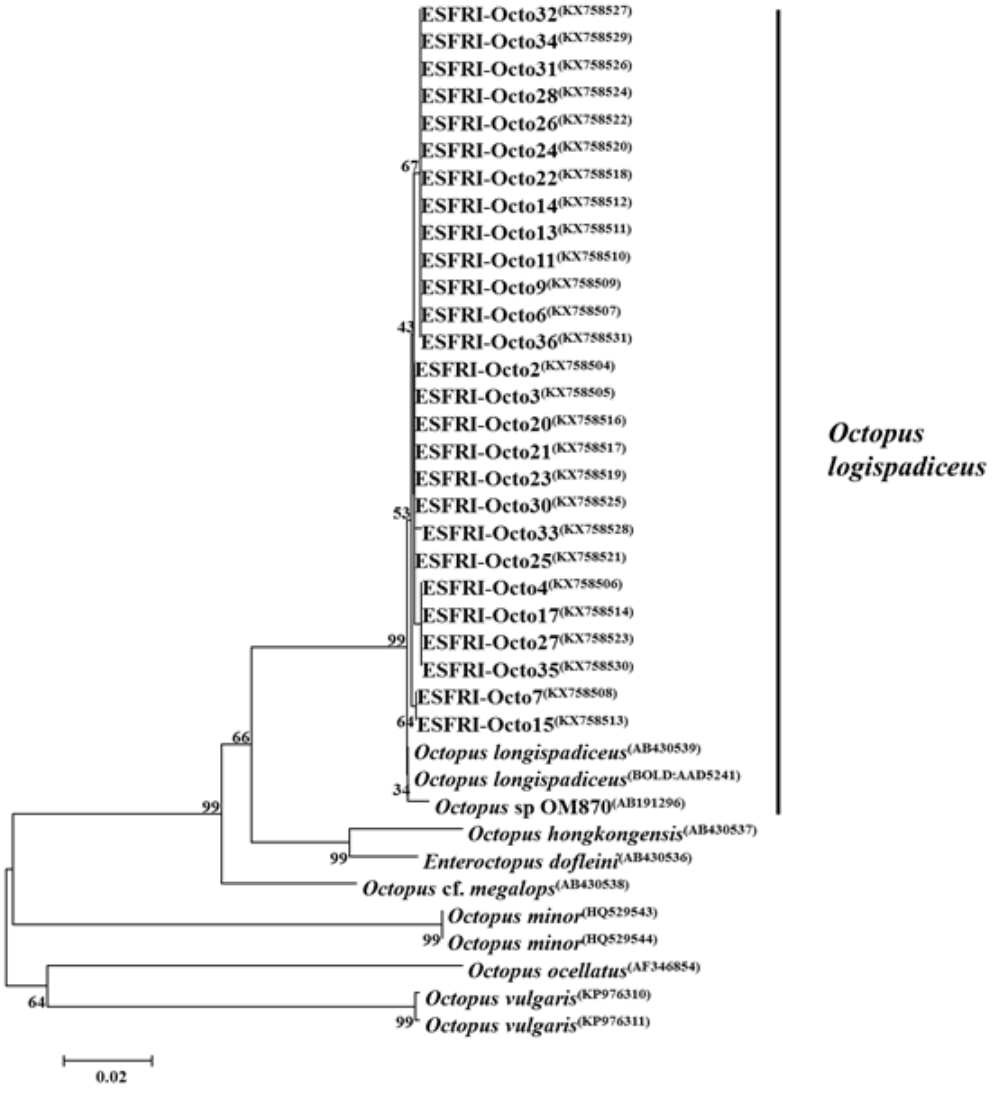

Fig. 6. Neighbor-joining tree based on 634-bp sequences of the mtDNA *COI* gene of *Octopus longispadiceus* collected from the East Sea of Korea and those of other Octopodidae species. Superscripts indicate the accession numbers in GenBank and BOLDSYSTEMS.

in the present study; length of the right third arm was 74%-77% of the longest arm in *O. longispadiceus* vs 74% in the present study; dominant arm formula: I > II > III > IV in *O. longispadiceus* vs I > II > III > IV in the present study; and the shapes of the terminal organ and ligula are consistent.

According to Gleadall (1993), *O. longispadiceus* is very similar to *O. yendoi* (Sasaki, 1929) collected from Cape Clonard, Korea. Sasaki (1929) mentioned the differences between *O. longispadiceus* and *O. yendoi* as follows: presence of 'Wart' above eye in *O. longispadiceus* vs absence in *O. yendoi*; 'C' or '6' shape of terminal organ in *O. longispadiceus* vs straight in *O. yendoi*; longer right third arm than left third arm in *O. longispadiceus* vs shorter in *O. yendoi*. According to Sasaki (1929), morphological characteristics of present specimens agree well with those of *O. yendoi*. However, Gleadall (1933) suggested that the differences are intra-specific variation or changes as the animals grow. For example, the 'cirrus' (or wart) above the eye is considered characteristic of *O. longispadiceus* (Sasaki 1917), but Gleadall (1993)

mentioned that only one specimen among four syntypes of *O. longispadiceus* had a cirrus above the eye.

A molecular analysis supported our morphological results. In this study, we amplified 660-bp sequences of the mtDNA *COI* region from 27 specimens and compared 634-bp of these sequences with those from the genus *Octopus* available in the NCBI and BOLDSYSTEMS databases. The genetic distances among our specimens were 0.000-0.003, so they were all the same species. Kaneko *et al.* (2011) analyzed the sequences of the mtDNA *COI* region of shallow water octopuses in Japan and adjacent waters including *O. longispadiceus* collected from Seto Inland Sea. In this analysis, the genetic distance between present specimens and *O. longispadiceus* from Japan was the smallest (d = 0.002-0.003) (Fig. 6).

Kim and Chun (2010) suggested that there are five species in the genus *Octopus* in Korea. In this study, *O. longispadiceus* was readily distinguishable from *O. minor* (Korean name: Nag-ji) by the arm length formula (I > II > III > IV in *O. longispadiceus* vs I > II > III = IV in *O. minor* with twice the length of the third or fourth arms). *Octopus ocellatus* (Korean name: Ju-ggu-mi) differed distinctly from *O. longispadiceus* by the presence of an ocellus on the lateral body (absent in *O. longispadiceus*), and differed from *O. vulgaris* (Korean name: Cham-mun-eo) by the arm length formula and white spots on the mantle (III > II > IV > I or III = II > IV > I and a diversely patterned groove on the mantle in *O. vulgaris*). *Octopus longispadiceus* was readily distinguishable from *O. dofleini* (Korean name: Dae-mun-eo), which is distributed only in the East Sea of Korea, by the white spots on its mantle (wrinkled parallel longitudinal ridges on the mantle in *O. dofleini*). Finally, *O. longispadiceus* was distinguishable from *O. megalops* (Korean name: Nun-keun-nag-ji) by length of right third arm in male (74% of the longest arm in *O. longispadiceus* vs 54% in *O. megalops*), absence of cirrus above the eye (presence in *O. megalops*) (Taki, 1964; Jereb *et al.*, 2016).

In this study, we identified our specimens as *Octopus longispadiceus* based on its morphological and molecular characters, and propose the new Korean name “Bal-mun-eo” for this species.

Summary

Fifty-five specimens, belonging to genus *Octopus*, were collected from the East Sea and identified as *O. longispadiceus* based on the following morphological characters: long right third arm (74% of the longest arm) and the presence of enlarged suckers in mature male, small white spots on mantle, head, and arms, and no cirrus above the eye. A molecular analysis supported the morphological results, and the genetic distance between present specimens and *O. longispadiceus* collected from Japan was the smallest. This species has not been reported previously from Korea, therefore, we propose the new Korean name “Bal-mun-eo” for this species.

Acknowledgments

This work was supported by a grant from the National Institute of Fisheries Science of Korea (R2016031).

REFERENCES

Cuvier, G. (1979) Tableau elementaire de l'histoire naturelle des animaux. 710 pp. Paris.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotech.*, **3**: 294-299.

Gleadall, I. G. (1993) Identification of the long-ligula octopuses of Japan: a status report.

Gray, J.G. (1849) Catalogue of the Mollusca in the collection of the British Museum. 1. Cephalopoda Antepedia. 164 pp. British Museum, London.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.*, **41**: 95-98.

Hoyle, W.E. (1885) Diagnosis of new species of Cephalopoda collected during the cruise of H.M.S. "Challenger"-I. Octopoda. *Annals and Magazine of Natural History*, **15.87**: 222-236.

Jereb, P., Roper, C.F.E., Norman, M.D. and Finn, J.K. (2016) Cephalopods of the world: an annotated and

illustrated catalogue of cephalopods species known to date, volume 3. Octopods and Vampire squids. Food and agriculture organization of the United, Rome.

Kaneko, N., Kubodera, T. and Iguchi, A. (2011) Taxonomic study of shallow-water octopuses (Cephalopoda: Octopodidae) in Japan and adjacent waters using mitochondrial genes with perspectives on octopus DNA barcoding. *Malacologia*, **54**: 97-108.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*., **16**: 111-120.

Kim, Y.H. and Chun Y.Y. (2010) Korean name of cephalopods in Korea. orean *J. Malacol*., **26**: 171-175.

Kim, Y.H., Lee, D.W., Hong, B.K. and Chun, Y.Y. (2008) First record of three species of Octopodidae and gonatidae, cephalopods in the East/Japan Sea. *J. Environ. Bio*., **29**: 581-584.

Nesis, K.N. (1987) Cephalopods of the worlds. 351 pp. T.F.H. Publications, New Jersey.

Norman, M.D. and Hochberg, F.G. (2005) The current state of octopus taxonomy. Phuket mar: *biol. Cent. Res. Bull*., **66**: 127-154.

Okutani, T., O'Dor, RK & T. Kubodera (eds): Recent advances in Fisheries Biology. Tokai University Press, Tokyo. 145-158.

Robson, G.C. (1929) A monograph of the recent cephalopoda. Part I. Octopodinae. 236 pp. British Meseum, London.

Sasaki, M. (1917) Notes on the cephalopoda-Ii. Diagnoses of four new species of *Polypus*, A*nnotationes Zoologicae Japonenses*, **9**: 364-367.

Sasaki, M. (1920) Report on cephalopods collected during 1906 by the United States Bureau of Fisheries steamer Albatross in the northwestern Pacific. Proceedings of the United States National Museum, Smithsonian Institution Press, *Washington*., **57**: 63-203.

Sasaki, M. (1929) A monograph of the dibranchiate cephalopods of the Japanese and adjacent waters. Journal of College of Agriculture. 357 pp. Hokkaido Imperial University, Hokkaido.

Takeda, R. (2003) A redescription and biological characteristics of *Octopus longispadiceus* (Sasaki, 1917) in the western part of the Sea of Japan. *VENUS*, **62**: 29-38.

Taki, I. (1964) On eleven new species of the cephalopoda from Japan, including two new genera of Octopodinae. *J. Fac. Fish Anim. Hush*., **5**: 277-343.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. Mega5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*., **28**: 2731-2739.

Wülker, G. (1910) Über japanische cephalopoden: Beiträge zur kenntnis der systematik und anatomie der dibranchiaten. Abhandlungen der mathematische-physikalische Klasse der Koeniglich Bayerischen Akademie der Wissenschaffen 3. 77 pp.