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Original Article

# First record of the hawksbill turtle (*Eretmochelys imbricata*, Reptilia: Testudines: Cheloniidae) from South Korea

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A B S T R A C T

To conserve national biodiversity, enlisting and detailed morphological description of unrecorded species is critical. Here, we, for the first time in Korea, report and describe detailed morphological characteristics of two juvenile hawksbill turtles, *Eretmochelys imbricata*, captured from Seongsan-eup, Seogwipo-si, Jeju-do and Geumnam-myeon, Hadong-gun, Gyeongsangnam-do, South Korea on 4 August 2016 and 19 December 2016, respectively. Both specimens had a narrow head, pointed beak, four pairs of costal scutes, and four pairs of poreless inframarginal scutes, which are typical morphological characteristics of *E. imbricata*. Phylogenetic analysis of mtDNA control region sequences of a specimen confirmed again that the specimens are *E. imbricata*. With this addition of *E. imbricata* ( Mae-bu-ri-ba-da-geo-buk in Korean), four species of sea turtles have now been reported from Korean waters.



## Introduction

Of the more than 12,000 species and subspecies of extant reptiles, about 100 have re-entered the ocean (Rasmussen et al 2011). Among them are seven species of sea turtles, and they form a monophyletic group comprising two families of Dermochelyidae (one species) and Cheloniidae (six species within five genera) under the suborder Cryptodira (Werneburg and Sanchez-Villagra 2009).

These seven species include the loggerhead sea turtle (*Caretta caretta*), green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricata*), Kemp's ridley sea turtle (*Lepidochelys kempii*), olive ridley sea turtle (*Lepidochelys olivacea*), flatback turtle (*Natator depressus*) in the family Cheloniidae, and leatherback sea turtle (*Dermochelys coriacea*) in the family Dermochelyidae (Márquez 1990; Pritchard and Mortimer 1999; Gomez and Miclat 2001). Sea turtles are found primarily along tropical coasts, but some turtles are also well-known for their long journeys, resulting in finding them in other oceans (Rasmussen et al 2011). In East Asia, which includes China, Taiwan Province of China (Taiwan), Japan, and Korea, five sea turtle species, one in the Dermochelyidae and four in the Cheloniidae have been listed (Zhao and Alder 1993; Goris and Maeda 2004; Chan et al 2007; Cheng and Chen 1997; Mortimer and Donnelly 2008; Wallace et al 2010; Kim et al 2017).

Up to date, green, loggerhead, and leatherback sea turtles have been reported from Korean coastal areas (Hironobu 1936a, 1936b; Won 1971; Kang and Yoon 1975; Kim and Han 2009); however, anecdotal report has only been made on hawksbill turtles for their rare observation and the species is not officially enlisted in the reptile list of Korea yet (Jung et al 2012, The Korean Society of Herpetologists). These days, individual numbers of most sea turtle species are dramatically reduced and all species are in either threatened or endangered status on a global level (Lutcavage et al 2017). Especially hawksbill turtle has been classified as critically endangered since 1996 (Mortimer and Donnelly 2008). To protect these vulnerable sea turtle species, including hawksbill turtle, the Ministry of Oceans and Fisheries of Korea has designated them as "Marine Organisms Under Protection" in 2015 (MOF 2015). Therefore, an official report of the hawksbill turtle, in Korean waters, is being greatly required. Here, for the first time, we officially report

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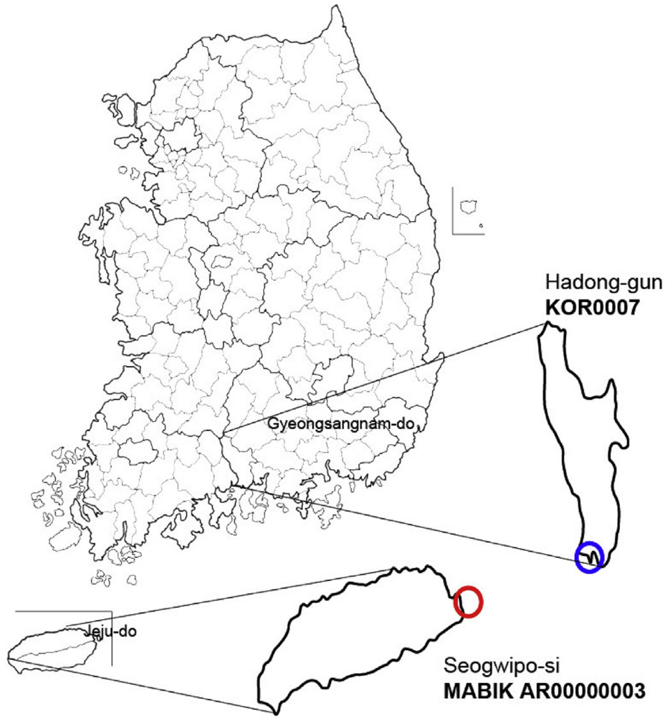


**Figure 1.** Location of hawksbill turtle (*Eretmochelys imbricata*) observed around Korean peninsula.

and provide morphological details of the hawksbill turtle (*E. imbricata*), collected from Korean waters.

## Material and methods

### *Collecting the specimen and morphological study*

A dead specimen, due to collision with ship, was collected from Sinyang-ri, Seongsan-eup, Seogwipo-si, Jeju-do (33° 26′N, 126° 55′E) on 4 August 2016 by a staff of Hanwha Aqua Planet Jeju, donated to the National Marine Biodiversity Institute of Korea (MABIK) and deposited into the herpetological collection there (MABIK AR00000003). We collected its tissue samples for genetic analysis and measured physical characteristics at MABIK.

The other specimen was rescued from by-catching in a setnet at Noryang-ri, Geumnam-myeon, Hadong-gun, Gyeongsangnam-do (34° 56′N, 127° 51′E) on 19 December 2016 by staffs of Hanwha Aqua Planet Yeosu, the institution specialized in rescuing and treating marine animals in South Korea. After measuring physical characteristics and veterinarian examination of the health condition of the specimen, the turtle was released at the by-catching site on 29 August 2018 (KOR0007, serial number used for the sea turtles that are released into the ocean in South Korea). Unfortunately, we could not collect any blood or tissue samples for genetic analysis (Figure 1).

Throughout surveys of museums in South Korea, we have found several preserved *E. imbricata*, but we did not include those in this study because basic information such as collector and collecting location were not presented.

We measured length parameters using Mantax Blue and vernier calipers (Hanglof, Sweden) and vernier caliper (Digital caliper, Mitutoyo Korea, Seoul, Korea), while body weights were measured using a digital balance (DL-100; CAS-Korea, Yangju, Korea) to the nearest 500 g. Because of damaged condition, we could not measure some parameters such as head size, body weight, and characteristics of some scutes in the first specimen. We morphologically classified the specimens following Márquez (1990), Pritchard and Mortimer (1999), Gomez and Miclat (2001), and Wyneken (2001). Photographs used in this study were taken with a digital camera (D810, Nikon; Tokyo, Japan) at MABIK and Hanwha Aqua Planet Yeosu, and only brightness and contrast of the photographs were adjusted in Photoshop (ver. 12.0).

### *DNA extraction, PCR, and phylogenetic analysis of mtDNA control region genes*

We extracted whole genomic DNA from hind leg muscle tissues of the first dead specimen, caught at Sinyang-ri, using a QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's instruction. To amplify the mtDNA control region (tRNA-Pro gene, partial sequence; and D-loop, partial sequence), we used the primer set with the forward primer LCM15382 (5'-GCTTAACCCTAAAGCATTGG-3') and reverse primer H950 (5'-GTCTCGGATTTAGGGGTTT-3') which was previously developed and used in *E. imbricata* (Abreu-Grobois et al 2006). PCR was performed with the AccuPower PCR Premix (Bioneer, Korea) and the reaction conditions were as follows: total volume 20 $\mu\ell$; template gDNA 1 $\mu\ell$; primers 2 $\mu\ell$ (10 $\mu$M); preheating at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 52°C for 60 s, and extension at 72°C for 90 s, with a final extension at 72°C for 10 min. We verified PCR products on 1.5% agarose gel by electrophoresis and purified the products using an AccuPrep® PCR Purification Kit (Bioneer, Daejeon, South Korea). Purified products were bidirectionally sequenced at the Macrogen Sequencing facility (Macrogen, Seoul, South Korea) using the same PCR primer set.

To construct the phylogenetic tree, we additionally downloaded 12 comparable sequences of *E. imbricata* from GenBank (512 ~ 513bp, accession numbers indicated in Figure 4). A total of 14 sequences were aligned with GENEIOUS v.9.1 software (Biomatters Ltd, Auckland, New Zealand) including outgroup taxa *Caretta caretta* (AB485804). Phylogenetic analyses were conducted using

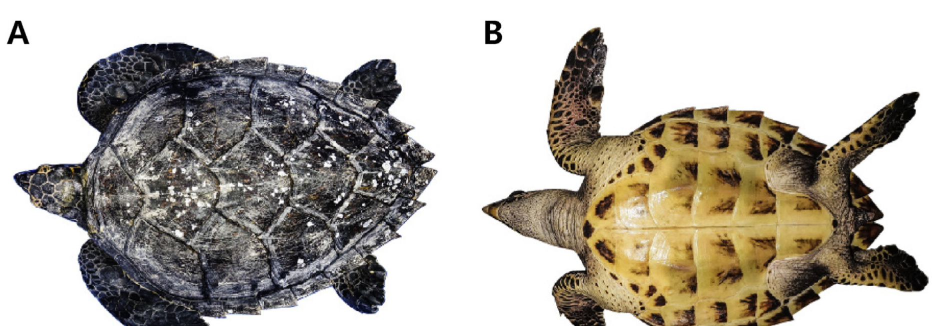


**Figure 2.** Hawksbill turtle (*Eretmochelys imbricata*) caught at Geumnam-myeon, Hadong-gun, Gyeongsangnam-do on 19 December 2016: A, dorsal surface; B, ventral surface.

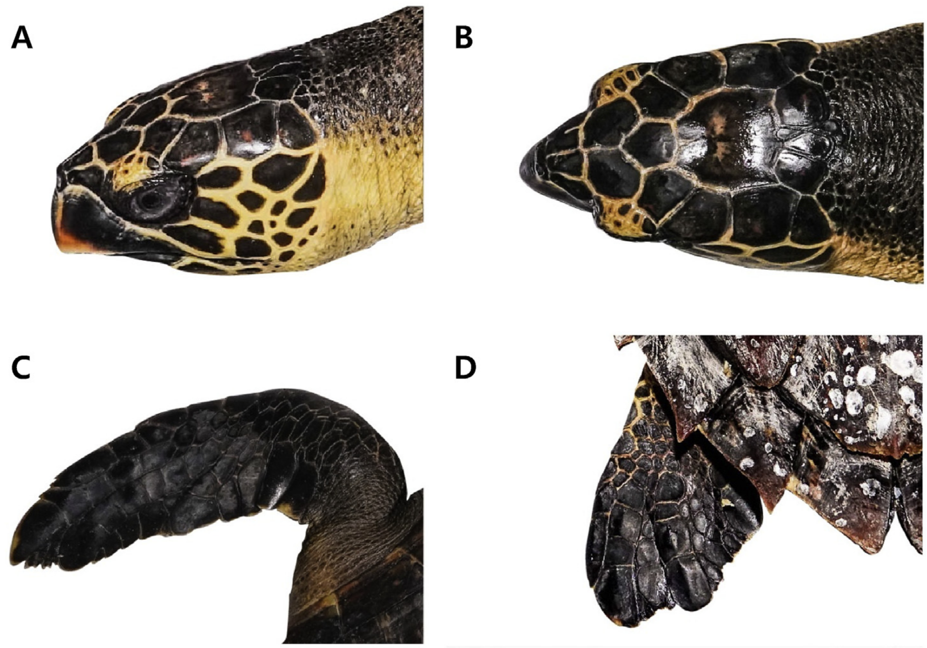


**Figure 3.** Hawksbill turtle (*Eretmochelys imbricata*) caught at Geumnam-myeon, Hadong-gun, Gyeongsangnam-do on 19 December 2016: A, lateral surface of the head; B, dorsal surface of the head; C, left forelimb; D, left hindlimb.

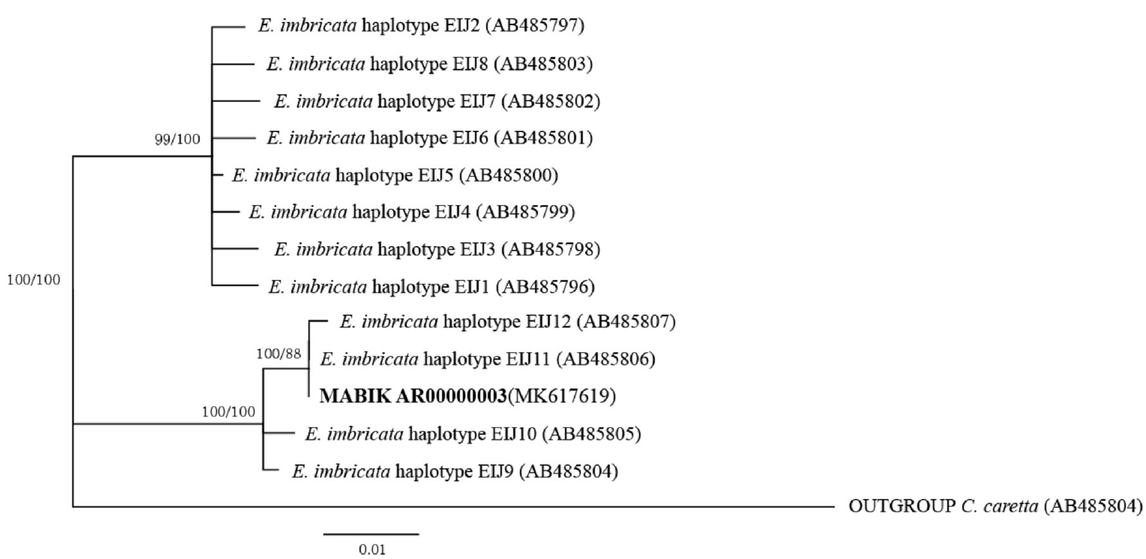


**Figure 4.** Maximum likelihood and Bayesian inference tree based on the mtDNA control region (tRNA-Thr gene, partial sequence; tRNA-Pro gene, complete sequence; and D-loop, partial sequence) of *Eretmochelys imbricata* collected at Geumnam-myeon, Hadong-gun, Gyeongsangnam-do, South Korea and other 12 *E. imbricata* sequences. We used the sequence of *Caretta caretta* as an outgroup taxon. The GenBank accession number of each sequence is presented after the each haplotype name of the species. On each branch, the maximum likelihood bootstrap value and Bayesian posterior probability are denoted.

maximum likelihood (ML) methods using PAUP 4.0a (Swofford 2002) and Bayesian inference (BI) using MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003). To infer a suitable model of sequence evolution for ML and BI methods, we selected the HKY model (ti/tv ratio: 3.6461, base frequencies: A = 0.3409, C = 0.1854, G = 0.1406, and T = 0.3331) using jModelTest 2.1.4 (Darriba et al 2012). Heuristic searches for ML analyses were conducted with 1000 replicates and stepwise addition starting with tree-bisection-reconnection branch swapping. Bayesian inference was implemented for 1,000,000 generations and sampling was done every 1000$^{th}$ generation until the average standard deviation was <0.01. The first 20% of trees were discarded as "burn-in".

## Systematic accounts

Order Testudines Batsch 1788
Family Cheloniidae Oppel 1811
[1]Genus *Eretmochelys* Fitzinger 1843

1 매부리바다거북속(Maeburibadageobuksok)

[2]*Eretmochelys imbricata* (Linnaeus 1766)

*Testudo imbricata* Linnaeus 1766: 350, Shaw & Nodder 1797: 287, Cuvier 1831: 15
*Chelonia pseudo-mydas* Lesson 1834 (in Bélanger),
*Chelonia imbricata* Duméril & Bibron 1835: 547
*Eretmochelys squamata* Agassiz 1857: 382
*Caretta rostrata* Girard 1858: 446
*Onychochelys kraussi* Gray 1873: 33
*Chelonia radiata* Cuvier 1829, Boulenger 1889
*Eretmochelys squamosa* Garman 1908: 9, Stejneger 1907: 511
*Caretta squamata* Swinhoe 1863: 221, Barbour 1918: 489
*Eretmochelys imbricata* Agassiz 1857, Stebbins 1985: 107, Engelmann et al. 1993, Glaw & Vences 1994: 229, Cogger 2000: 182, Trape, Chirio & Trape 2012, Spawls et al. 2018: 42, Conant & Collins 1991: 76
*Eretmochelys imbricata squamata* Carr 1942, Meirte 1999
*Eretmochelys imbricata imbricata* Mertens & Müller 1928, Crother 2000
*Eretmochelys imbricata bissa* Rüppell 1835, Smith & Taylor 1950, Stebbins 1985: 107, Crother 2000

*Description*

*Carapaces*

Nearly elliptical; SCW (straight carapace width) length 79.8% (Hadong-gun specimen) and 79.3% (Seogwipo-si specimen) of SCL max (maximum straight carapace length) (Figure 2A). Carapace with nuchal scute, 5 vertebral scutes, 4 pairs of costal scutes (the first not touching the nuchal scute), 11 (Hadong-gun specimen) and 12 (Seogwipo-si specimen) pairs of marginal scutes, and 1 pair of supracaudal scutes (Figure 2A).

*Plastron*

With intergular scute and two paired gular, humeral, pectoral, abdominal, femoral, and anal scutes; plastron bridges; four pairs of inframarginal scutes without pores (Figure 2B).

*Head*

(Hadong-gun specimen only) Medium sized, narrow, with pointed beak; length 28.2% of SCL max; with two pairs of prefrontal scales, three pairs of temporal scales (Figure 3A), and four pairs of postocular scales (Figure 3B).

*Limbs: 2*

Claws on each flipper (Figure 3C, 3D).

*Color*

Heavy melanistic form; scales of head with creamy or yellow margins; dorsal carapace with amber ground color and black stripes (Figure 2). Physical characteristics are shown in Table 1.

We obtained a total of 742 bp sequence of mtDNA control region from our specimen (MABIK AR00000003). In the phylogenetic analysis, our sequence was well fitted into a previously known *E. imbricata* group (Figure 4. The haplotype of our sequence was completely identical to the previously known haplotype EIJ11 (516 bp, GenBank accession number: AB485806), which was reported from the *E. imbricata* caught at Yaeyama Islands, Japan (Nishizawa et al. 2010).

[2] 매부리바다거북(Maeburibadageobuk)

**Table 1.** Physical characteristics of the two hawksbill turtles (*Eretmochelys imbricata*) caught in Korea in 2016.

| Physical parameters (cm) | Measurements (cm) | |
|---|---|---|
| | MABIK AR00000003 (Juvenile, 4 August 2016) | KOR0007 (Juvenile, 19 December 2016) |
| Maximum head length (HL) | - | 12.24 |
| Maximum head width (HW) | - | 7.61 |
| Maximum straight carapace length (SCL max) | 38.8 | 43.4 |
| Maximum curved carapace length (CCL max) | 43.4 | 45.3 |
| Minimum straight carapace length (SCL min) | 36.5 | 41.2 |
| Minimum curved carapace length (CCL min) | 41.2 | 43.2 |
| Straight carapace width (SCW max) | 31.0 | 34.4 |
| Curved carapace width (CCW min) | 37.1 | 40.1 |
| Curved plastron length (CPL max) | 30.0 | 34.7 |
| Curved plastron width (CPW max) | 27.5 | 30.5 |
| Plastron-to-vent length (PVTL) | - | 6.32 |
| Vent-to-tip length (VTTL) | 1.3 | 2.88 |
| Body weight (BW) | - | 11.8 |

- Unable to measure.

## Discussion

In general, chelonid sea turtles have carapace and plastron covered with scutes unlike dermochelyid sea turtles (Pritchard and Mortimer 1999; Gemez and Miclat 2001). Therefore, our specimens having pointed beak and strongly imbricated scutes, which are characteristics, only known in chelonid turtles suggest that the specimens are chelonids. In addition, the number and shape of each scute and scale in different body parts exactly matched to previously known typical morphological characteristics of *E. imbricata* (Márquez 1990; Pritchard and Mortimer 1999; Gomez and Miclat 2001; Wyneken 2001).

In the phylogenetic analysis of mtDNA control regions, phylogenetic location of our specimen was well fitted into the known *E. imbricata* group. Furthermore, the obtained sequence of our specimen was identical to EIJ11 haplotype of *E. imbricata* (AB485806, Nishizawa et al 2010), which is previously known. There is a clear genetic difference between Pacific and Atlantic hawksbill turtle populations (Reece et al 2005, Nishizawa et al 2010), although their genetic diversity in Pacific populations is not well known. Therefore, hawksbill turtles, found in both Japanese and Korean waters, should be in the same unit of East Asia Regional Management Units (Wallace et al 2010).

Taken together, both our morphological and molecular data strongly suggest that sea turtles collected at Jeju-si and Hadong-gun (MABIK AR00000003, KOR0007) were *E. imbricata*. Therefore, our report is the first official report of *E. imbricata* in Korea. As the result, four species of sea turtles (*Dermochelys coriacea, Eretmochelys imbricata, Chelonia mydas,* and *Caretta caretta*) have been reported from Korean waters.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Acknowledgments

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