ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

Korean J. Environ. Biol. 35(4) : 521~525 (2017) https://doi.org/10.11626/KJEB.2017.35.4.521

〈Original article〉

New Record of a Marine Algal Species, *Ahnfeltiopsis linearis* (Phyllophoraceae, Gigartinales), in Korea

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Abstract - A marine algal species belonging to Gigartinales was collected from Geoje, Korea. This shares the generic features of *Ahnfeltiopsis*, such as multiaxial thalli with a compact and pseudoparenchymatous medulla, densely cytoplasmic secondary medullary cells around immersed cystocarps with a carpostome, and is distinct from similar species within the genus by a combined feature of small (up to 4 cm tall) and tuft thalli, compressed to subcompressed branches except for ultimate branchlets and base of main axes, cartilaginous in texture, dichotomous branches, rarely produced proliferations, absence of hypha-like filament in the medulla and internal cystocarps with a carpostome. In phylogenetic tree based on *rbc*L sequence, the Korean species nests in the same clade with *Ahnfeltiopsis linearis*. The genetic distance between both sequences within the clade was 1.5%, considered to be within the intra-species range for the genus. This morphological and molecular evidence confirms the Korean alga to be identified as *A. linearis* originally described from California. This is the first record of *A. linearis* in Korea.

Keywords : the first record, morphology, molecular evidence, *rbc*L sequence

INTRODUCTION

Introduced species are a major problem throughout the world`s oceans because of disturbance of ecosystem and the resulting significant economic losses (Carlton 1996, 2000; Maggs and Stegenga 1999; Occhipinti-Ambrogi and Sheppard 2007). Unlike this, the importance of native species has recently been emphasized as industrial resources with the Convention on Biological Diversity. Therefore, this kind of study of flora and fauna has been carried out extensively in Korea.

Major historical collections and floristic studies on Korean marine algae were started in the late 1950s and early 1960s, and were published by Kang (1966). At that time, a total of 414 species of marine algae was listed in Korea. Since Kang (1966), many species have been added to the Korean marine algal flora (Lee and Kang 1986, 2002; Kim *et al*. 2013). In the past, these resulted mainly in floristic, phenological and ecological surveys based on morphology (Lee and Kang 1986, 2002). However, recently many researches have adopted molecular analysis (Lee *et al*. 2014; An and Nam 2015; Bustamante *et al*. 2015; Calderon *et al*. 2016). This has increased our understanding of Korean marine algae including cryptic species. Therefore, approximately 900 species are currently recorded in Korea (Kim *et al*. 2013).

During a survey of marine algal flora, a red algal species belonging to Gigartinales was collected from Geoje, located on the southern coast of Korea. This species was identified based on morphological and molecular analyses, and is newly recorded in Korea herein.

MATERIALS AND METHODS

Samples for the present study were collected from Geoje, Korea. All specimens were preserved in a 5-10% formalin-seawater solution, and pressed on herbarium sheets. A por-

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tion of the material was dried and preserved in silica gel for molecular analysis. Sections of the thallus were mounted in 20% corn syrup for permanent preparation.

Total genomic DNA was extracted from the sample preserved in silica gel using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Before extraction, dried material was crushed with liquid nitrogen using a mortar and pestle. Concentrations of extracted DNA were assessed using gel electrophoresis on a 1% agarose gel. Extracted DNA was used for amplification of the *rbc*L regions using published primers. PCR amplifications were performed in a TaKaRa PCR Thermal Cycler Dice with an initial denaturation step at 94°C for 5 min followed by 35 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min and a final extension at 72°C for 7 min. The reaction volume was 20 μL, consisting of 20 ng of genomic DNA, 2 μL of 10×PCR buffer, 2 μL of 200 μM dNTP, 1 μL of each forward and reverse primer, and 0.5 units of Taq polymerase (Takara Korea, Korea). Amplifications were examined using gel electrophoresis in a 1% agarose gel and amplified ITS region products were purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The PCR products were moved to Macrogen Sequencing Service for sequencing (Macrogen, Seoul, Korea). The PCR primers were also used for sequencing. It is as follows: *rbc*L (forward: 5′ GGAGGATTAGGGTCCGATTCC 3′, reverse: 5′ CTTCCGTCAATTCCTTTAAG 3′) (Lin *et al*. 2001).

Sequences for the *rbc*L region were aligned using BioEdit (Hall 1999). Phylogenetic analyses were performed using the maximum-likelihood (ML) method. Bootstrap values were calculated with 1,000 replications. *Rbc*L sequences of other species were obtained from GenBank. *Ahnfeltia plicata* and was used as an outgroup.

RESULTS AND DISCUSSION

***Ahnfeltiopsis linearis* (C. Agardh) P.C. Silva & DeCew 1992: 578.**

Type locality: Trinidad, Humboldt Co., California (Silva 1979).

Korean name: Sil-bu-chaet-sal nom. nov. (신칭: 실부챗살).

Specimens examined: NIBRRD0000001653, PKNU0000134850, PKNU0000134852 (Nambu, Geoje: 08.v.2015).

Habitat: Epilithic near the lower intertidal.

Morphology: Thalli 2–4 cm high, dense tuft, compressed to subcompressed but subterete to terete in ultimate branchlets and at base of main branch, fan-shaped, brown to yellow in color, cartilaginous in texture, attached to substratum by discoid holdfast (Fig. 1A–C); erect axes multiaxial, issuing dichotomous or subdichotomous branches in same plane; branches with rounded or blunt apex, 1–3 mm wide, 100–200 μm thick; proliferations rare, arranged pinnately to irregularly; cortex consisted with small, round to elongate and pigmented cells, three to six cell layers thick, 2–4×3–6 μm (Fig. 1D); medulla pseudoparenchymatous, compact, consisting of round to ellipsoidal cells with size of 20–40×30–60 μm in transverse section of branches, without hypha-like filaments, 100–200×60–90 μm (Fig. 1D); nonprocarp with 3-celled carpogonial branch (Fig. 1E); auxiliary cell produced prior to fertilization; carposporophytes producing masses of carposporangia with 200–300×300–400 μm in size; cystocarps formed at middle portion of branches, internally immersed in medulla, surrounded by some layers of densely cytoplasmic secondary medullary cells, with a carpostome, 10–20×60–70 μm (Fig. 1F). Male and tetrasporangial plants were not collected during the present study.

Ahnfeltiopsis, which involves 33 species distributed from temperate to tropical waters (Dawson 1954; Masuda 1993; Silva *et al*. 1996; Guiry and Guiry 2017) was erected based on internal cystocarps and a heteromorphic life history with upright unisexual gametophytes and a crustose tetrasporophyte (Silva and DeCew 1992; Masuda 1993). However, it was recently redefined by some combined features of female structure, such as cystocarp compactly immersed within the medulla, lateral arrangement of carposporangia connected to medullary cells, presence of heterokaryotic medullary cells, and secondary medullary cells around cystocarps and carpostome (Calderon and Boo 2016). In our specimens, densely cytoplasmic medullary cells, which are considered to be formed secondary, are found around cystocarp (Fig. 1F). The carpostome-like specialized pores are also observed in the internal cystocarps (Fig. 1F). Even though other female features adopted by Calderon and Boo (2016) are not confirmed, our Korean alga can be referred to *Ahnfeltiopsis* based on this observation. This is also sup-

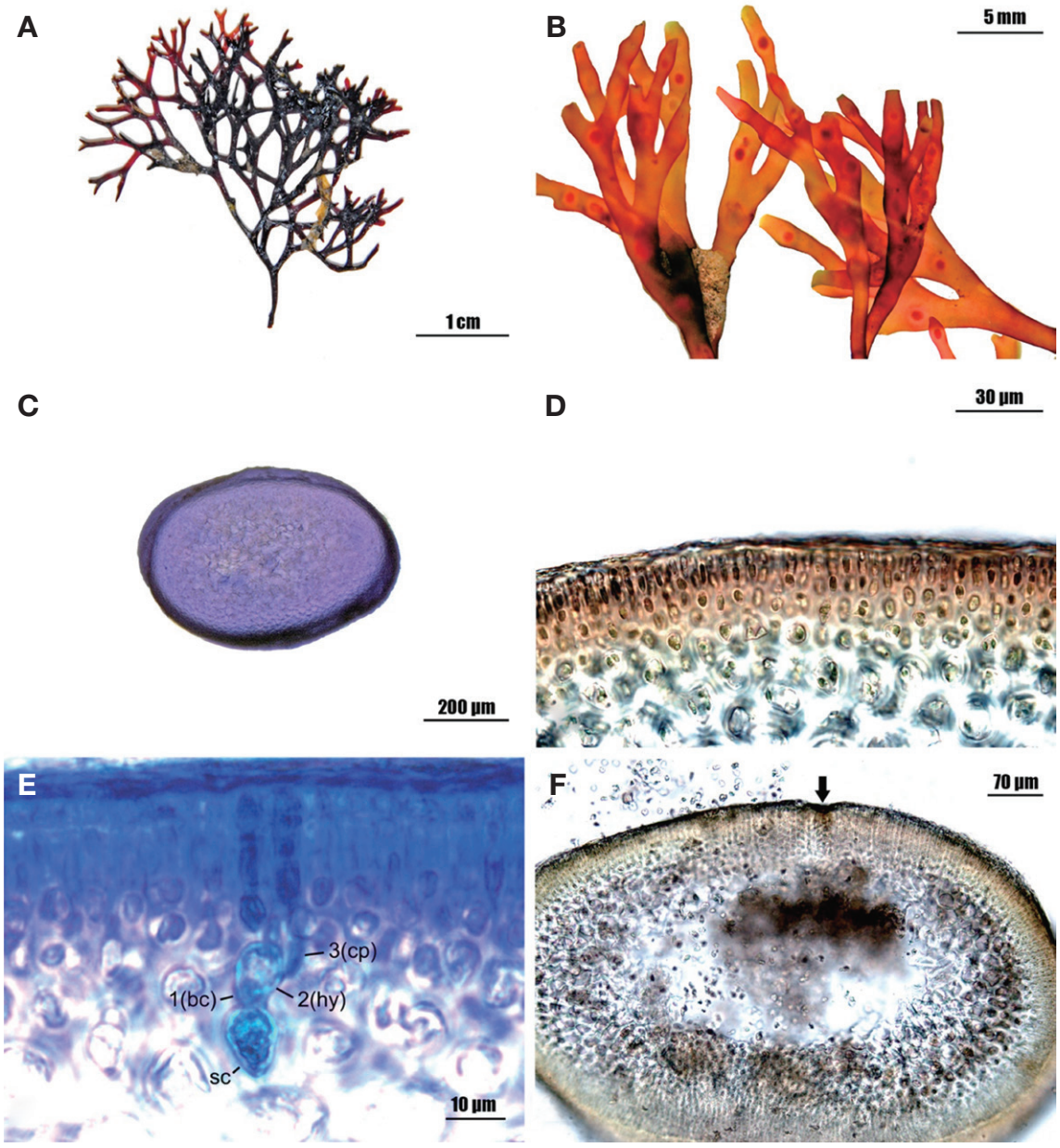

Fig. 1. *Ahnfeltiopsis linearis*. (A) Habit of the herbarium specimen (PKNU0000134852). (B) Details of the branches with cystocarps (red dots). (C) Transverse section of the subterete ultimate branchlet. (D) Cortex and medulla in a transverse section of the branches. (E) Three-cell carpogonial branch (sc, supporting cell; bc, basal cell; hy, hypogynous cell; cp, carpogonium). (F) Fully developed cystocarp with a carpostome (arrow).

ported by molecular analysis (Fig. 2). *Ahnfeltiopsis linearis* is the type of genus, and was originally described from California (Silva 1979). According to Abbott and Hollenberg (1976), this species appears to be distinct from other Californian species within the genus by having tuft thalli with many erect axes, relatively large size (up to 10−18 cm tall), ultimate branches not complanate and light tan to brownish purple in color. The Korean alga shares these features with the exception of a relatively small thallus size and the color, and is distinguished from the similar Korean *A. flabelliformis* (Kang 1966, 1968; Lee and Kang 1986, 2002; Boo and Ko 2012; Kim *et al*. 2013), by the features of proliferation and cystocarps. It rarely has proliferations and internal cystocarps with a carpostome, while in *A. flabelliformis* prolifer-

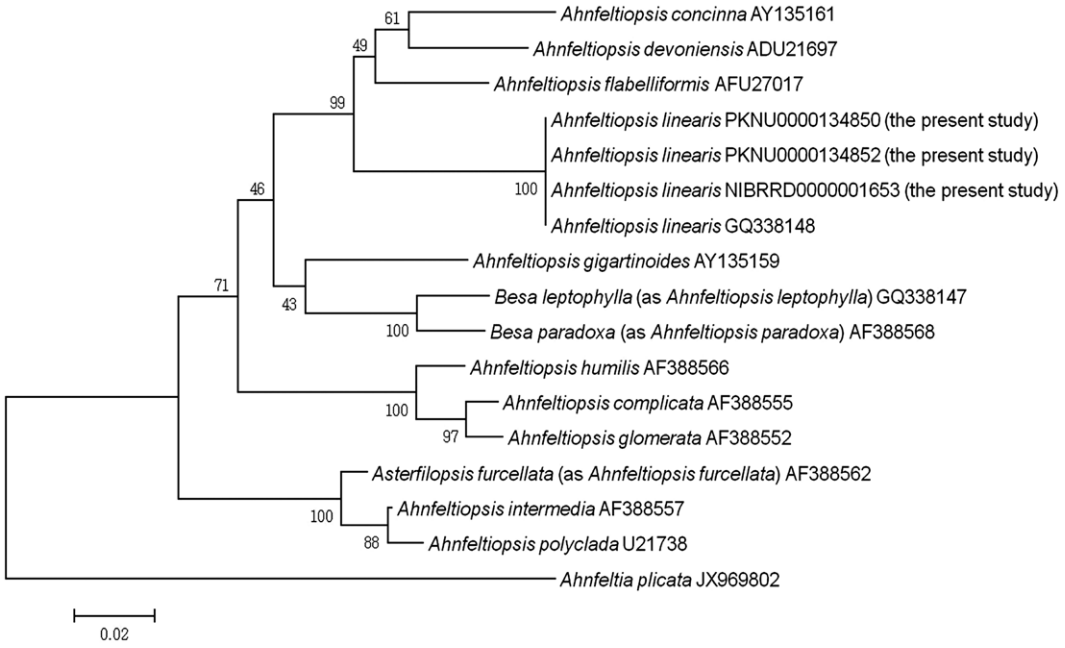

Fig. 2. Phylogenetic tree of the *Ahnfeltiopsis* species that is based on the *rbc*L sequences and was created using the maximum-likelihood method. The bootstrapped proportion values (1,000 replicate samples) are shown as branches. The scale bar indicates 0.02 substitutions/site.

ations are often found along the axis margin and cystocarps with multiple carpostomes are somewhat protuberant (Kang 1968; Masuda 1987; Abbott 1999; Lee 2008; Boo and Ko 2012).

In a phylogenetic tree based on *rbc*L sequence data (Fig. 2), the Korean alga nests in the same clade with *A. linearis*. The genetic distance between both sequences within the clade was calculated as 1.5%, considered to be within the intra-species range for the genus. In general, the value of interspecific divergence in the Gigartinaceae and Peyssonneliaceae within the Gigartinales varies from 2.8% to 16.5% (Hommersand *et al*. 1994; Kato *et al*. 2009).

This morphological and molecular evidence confirms that the Korean gigartinalean alga is identified as *Ahnfeltiopsis linearis* originally described from California, U.S.A. This is the first record of the species in Korea.

ACKNOWLEDGEMENT

This work was supported by a Research Grant of Pukyong National University (2017).

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Received: 18 October 2017
Revised: 20 November 2017
Revision accepted: 21 November 2017