

New record of *Chondrus retortus* (Gigartinales, Rhodophyta) in Korea

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Abstract: A marine red algal species was collected from Honghyeon-ri, Namhae located on the southern coast of Korea during a survey of marine algal flora. This alga shares the generic features of *Chondrus*, and is characterized by short and thin, canalculated and crisped fronds as *C. retortus*. Tetrasporangia are developed from shallow medullary cells on both sides of branches. In a phylogenetic tree based on *rbcL* sequences, the Korean alga nests in the same clade as *C. retortus* originally described from Japan. The genetic distance between both sequences within the clade was calculated as 0.0–0.1%. Based on the morphological and molecular data, the alga is identified as *Chondrus retortus*. This is the first record of *C. retortus* in Korean marine algal flora.

Keywords: *Chondrus retortus*, red alga, morphological and molecular data, Korea

INTRODUCTION

The red algal genus *Chondrus* (Gigartinales, Gigartinaceae) was typified with *Chondrus crispus* Stackhouse, which is distributed across the North Atlantic coast (Brodie *et al.* 1991; Hommersand *et al.* 1993, 1994). This genus is primarily characterized by lacking an envelope separating carposporangia from gametophytic tissues (Mikami 1965; Hommersand *et al.* 1993), and currently includes 17 species worldwide (Hommersand *et al.* 1993; Brodie *et al.* 1997; Guiry and Guiry 2020). These species are mainly distinguished by thallus shape and position of reproductive structures (Taylor and Chen 1994; Matsumoto and Shimada 2013).

Of these, *Chondrus verrucosus* Mikami (1965) was originally described from Inubosaki, Chiba Prefecture, Japan, based on *Chondrus ocellatus* Holmes f. *canaliculatus* Okamura (1932) with linear to cuneate, canalculated fronds. This species seems to be delimited from other *Chondrus* species by strongly canalculated fronds, verruciform cysto-

carps, and reproductive structures limited to distal portions of the thallus (Mikami 1965). According to Matsumoto and Shimada (2013), *C. retortus* is very similar to *Chondrus verrucosus* in morphology. In the present study, a species similar to *Chondrus verrucosus* and *C. retortus* was collected from the southern coast of Korea, and is newly recorded in Korean marine algal flora based on morphological and molecular analysis.

MATERIALS AND METHODS

Specimens for this study were collected from Honghyeon-ri, Namhae located in the southern coast of Korea. Morphological data were obtained from fresh, liquid-preserved and herbarium specimens. Liquid-preserved material was stored in a 10% solution of Formalin/seawater. Blades dissected from the cleared materials were hand sectioned, transferred to a slide with distilled water, and mounted in pure glycerin. Measurements are given as

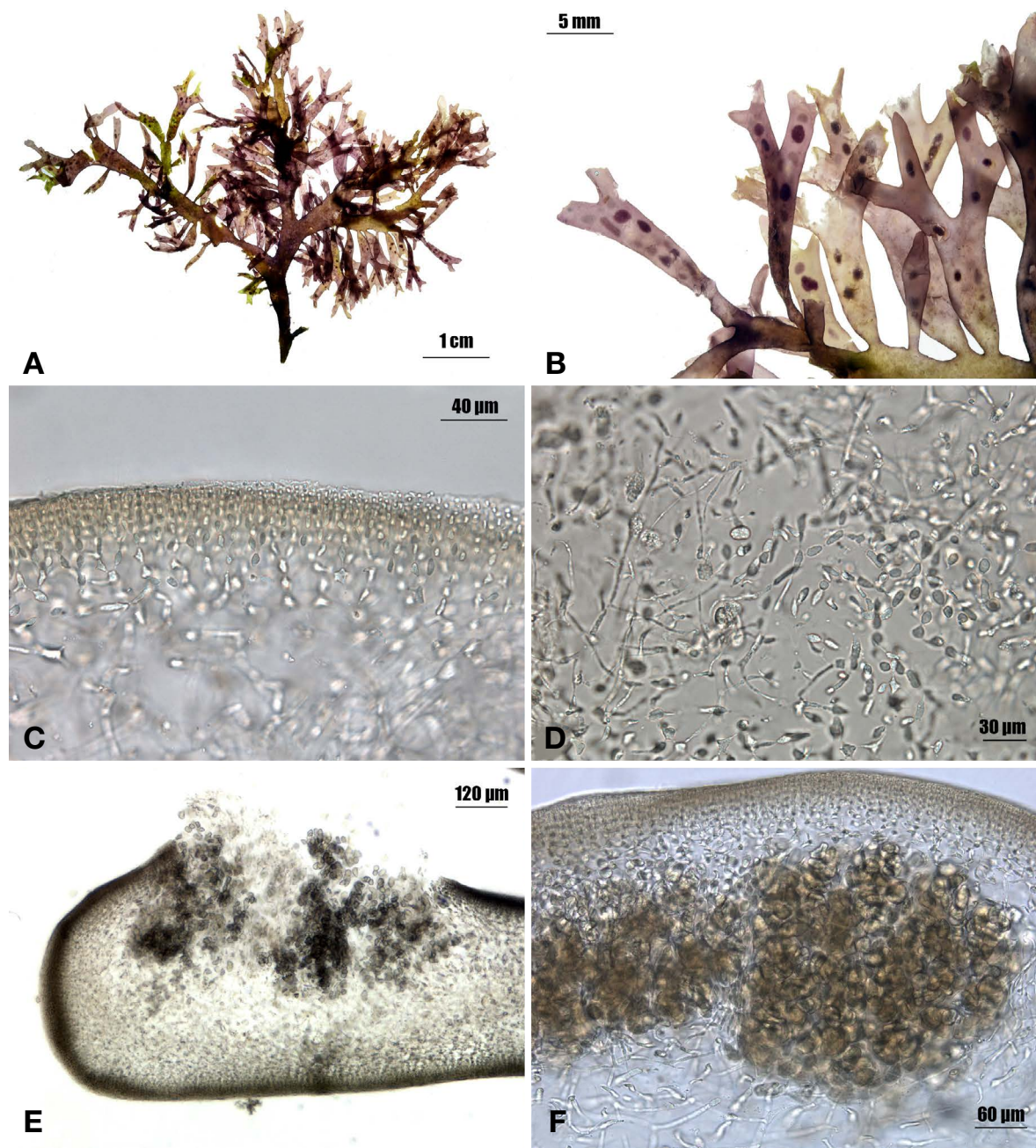


Fig. 1. *Chondrus retortus*. A. Tetrasporangial plant. B. Details of branches with tetrasporangial sori scattered on both sides. C. Cortical cell layers. D. Medullary cells. E. Mature tetrasporangial sorus bulging from one side of branch. F. Mature tetrasporangia developed from swollen medullary cells.

width and length. For permanent slides, the glycerin was exchanged with 10–20% corn syrup.

Total genomic DNA was extracted from silica-gel-preserved sample using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's pro-

cedure. Before extraction, dried material was crushed with liquid nitrogen using a mortar and pestle. Extracted DNA was used for amplification of ribulose-1, 5-bisphosphate carboxylase large subunit (*rbcL*) regions. For *rbcL*, the gene was amplified in three overlapping parts with the primer

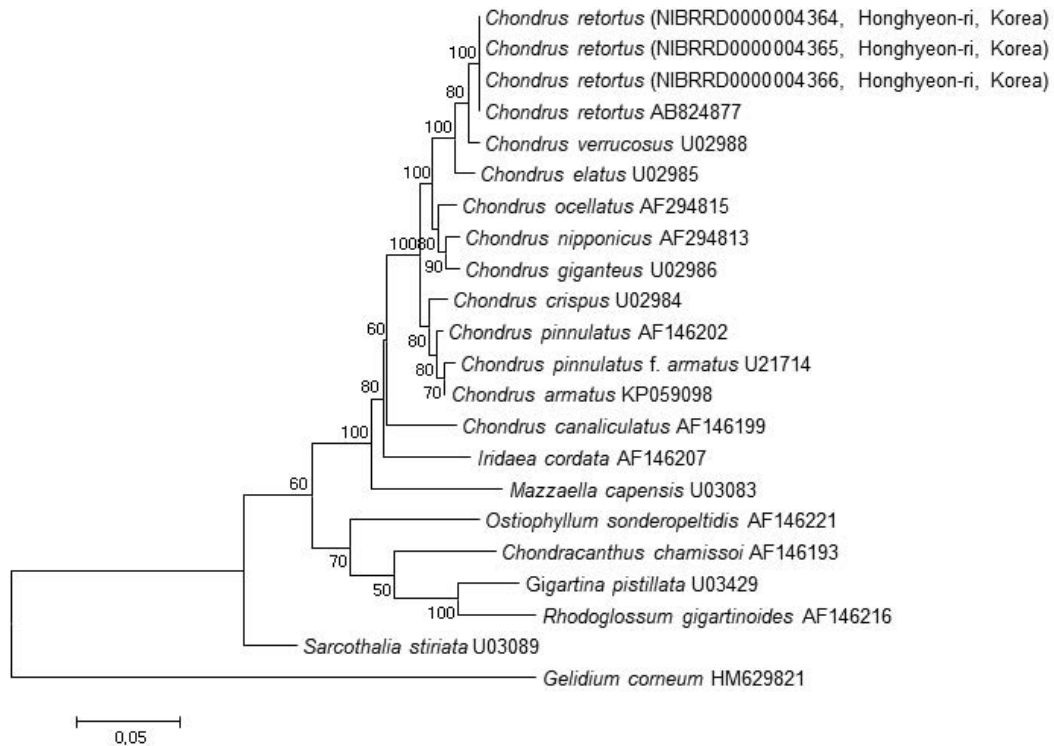


Fig. 2. Phylogenetic tree of *Chondrus* species obtained from maximum-likelihood method based on *rbcL* sequences. Bootstrap proportion values (1,000 replicates samples) are shown above branches. Scale bar = 0.05 substitutions/site.

pairs *Frbcl* start (5'-TGTGTTGTCGACATGTCTA-
ACTCTGTAGAAG-3') - R753 (5'-GCTCTTTCATA-
CATATCTTCC-3'), F492 (5'-CGTATGGATAAATTTG-
GTCG-3') - R1150 (5'-GCATTTGTCCGCAGT-
GAATACC-3'), and F993 (5'-GGTACTGTTGTAGG-
TAAATTAGAAGG-3') - *RrbcS* (5'-TGTGTTGCGGC-
CGCCCTTGTGTT AGTCTCAC-3') (Freshwater and
Rueness 1994). PCR amplifications were performed in
a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio Inc.,
Otsu, Japan). PCR was performed with an initial dena-
turation step at 94°C for 4 min, followed by 35 cycles of
1 min at 94°C, 1 min at 50°C, and 2 min at 72°C, with a
final 7-min extension at 72°C. The PCR products were
moved to MacroGen Sequencing Service for sequencing
(MacroGen, Seoul, Korea). Sequences for the *rbcL* region
were aligned using BioEdit (Hall 1999). Phylogenetic
analyses were performed using neighbor joining and max-
imum-likelihood methods. Bootstrap values were calculat-
ed with 1,000 replications. *RbcL* sequences of other species
were obtained from GenBank. *Gelidium corneum* (Hudson)
J.V. Lamouroux was used as an outgroup.

RESULTS AND DISCUSSION

Chondrus retortus K. Matsumoto & S. Shimada 2013

Korean name: Ae-gi-jin-du-bal-sa-chon nom. nov.

(신칭: 애기진두발사촌)

Type locality. Enoshima, Kanagawa Prefecture, Japan.

Specimens examined: NIBRRD0000004364–NIBRRD
0000004366 (Honghyeon-ri, Namhae; 25.x.2018).

Habitat: Epilithic near upper to lower intertidal.

Morphology: Thalli up to 5–10 cm high, compressed, thin,
somewhat canaliculated, crispy, yellowish to dark brown
in color, cartilaginous in texture, attached to substrate by
small discoid holdfast; main axes issuing numerous branch-
es and proliferations on margin and surface; proliferations
lanceolate, pinnately arranged, more or less constricted at
base, 5–10 mm in length, 1–2 mm in width; cortex 5–8 cell
layer thick, with 2–3 cell layer thick in outer cortex; inner
cortical cells ellipsoid to round in shape; medullary cells
densely arranged; tetrasporangial sori round to polygonal,
scattered on both sides of branches; tetrasporangia round
to ellipsoidal, developed from shallow medullary cells, 10–
30 µm in diam., divided tetrahedrally. Sexual plants were

not collected during the present study.

According to the original description (Matsumoto and Shimada 2013), *C. retortus* appears to be characterized by short, canalculated and crisped fronds, carposporangia developing among roundish medullary and tetrasporangial development from shallow medullary. The vegetative and tetrasporangial features are found in the Korean alga collected from Namhae (Fig. 1).

Chondrus retortus is readily distinguished from the similar species, *C. verrucosus*, by the cell shape of the medullary layer around young carposporangia (Matsumoto and Shimada 2013). In the former, the cells are roundish, while the latter has a linear form (Matsumoto and Shimada 2013). In addition, both species are also distinguishable from each other in some vegetative features including thallus size in the mature stage. *C. retortus* has thinner, somewhat canalculated and relatively small mature fronds rather than thicker, comparatively regularly canalculated and typical large mature fronds (Matsumoto and Shimada 2013).

The position of tetrasporangial sori is an important diagnostic feature among the Gigartinaceae, though it is parophyletic with respect to some genera (Mikami 1965; Kim 1976; Hommersand *et al.* 1999; Matsumoto and Shimada 2013). In *Chondrus*, the type species *C. crispus* has tetrasporangial sori filling the whole medulla, whereas in some species such as *C. elatus*, *C. ocellatus*, *C. verrucosus* and *C. yendoii*, they are developed from shallow medullary cells (Mikami 1965; Brodie *et al.* 1991; Fredericq *et al.* 1992; Matsumoto and Shimada 2013). *Chondrus retortus* also shows the same developmental features of tetrasporangial sori as *C. verrucosus* and *C. elatus* of the sister clade.

Although female plants were not collected during the present study, our Korean specimens can be identified as *C. retortus* based on the vegetative features. Their identity is supported by molecular data.

In general, the value of interspecific divergence in the Gigartinales varies from 2.8 to 16.5% (Hommersand *et al.* 1994; Kato *et al.* 2009). In the phylogenetic tree based on *rbcL* sequence, the Korean alga nests in the same clade as *Chondrus retortus* with a genetic distance of 0.0–0.1% (Fig. 2). Based on these morphological and molecular data, this species is identified as *Chondrus retortus*. This is the first record of *C. retortus* in Korean marine algal flora.

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