



New record of the genus *Neodilsea* and *N. yendoana* (Dumontiaceae, Gigartinales) in Korea

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Abstract

A marine algal species was collected from the southern coast of Korea. This alga is characterized by a large and cartilaginous thallus, a dichotomous branching pattern, compressed axes, rare proliferations near the apex, and cystocarps protruded toward surface. In a phylogenetic tree based on *rbcl* sequences, this species nests in the same clade as *Neodilsea yendoana* as a terminal clade of the genus *Neodilsea*. The genetic distance between both sequences within the clade was calculated as 0.2% considered to be in the intraspecific range within the genus. Based on the morphological and molecular data obtained in the present study, this Korean species is identified as *N. yendoana*. This is the first record of the genus *Neodilsea* and *N. yendoana* in Korea.

Key words: first record, Korea, morphology, *Neodilsea yendoana*, *rbcl* sequences, red algal species

INTRODUCTION

Neodilsea Tokida (1943), which was established based on *N. yendoana* from Japan, was separated from *Dilsea* Stackhouse by some reproductive characters, such as anatomical features of spermatangia, auxiliary cell branches, cystocarps, and tetrasporangia (Tokida 1943). However, it was known that both genera could be distinguished from each other only by a single feature, tetrasporangial position in cortical filaments: intercalary (or possibly terminal) in *Dilsea* vs. lateral in *Neodilsea* (see Bert 1966). Kraft (1978) and Hansen (1980) had a question on this use of a single diagnostic character for generic distinction. According to Lindstrom and Scagel (1987) and Lindstrom (1988, 1994), it is difficult to find characters to separate between the two genera. Recently, Tai et al. (2001) found that the recognition of *Dilsea* and *Neodilsea* as distinct

genera is tenuous in the molecular analyses. Nevertheless, *Neodilsea* is currently accepted as a distinct genus within Dumontiaceae Bory de Saint-Vincent (Saunders 2008, Guiry and Guiry 2015). Six species in this genus have been recognized worldwide (Guiry and Guiry 2015): *N. borealis* (I.A. Abbott) S.C. Lindstrom, *N. crispata* Masuda, *N. longissima* (Masuda) S.C. Lindstrom, *N. orientalis* N.G. Kloczcova, *N. tenuipes* Yamada & Mikami and *N. yendoana*.

A red algal species belonging to the Dumontiaceae was collected from the southern coast of Korea during a survey of indigenous species of marine algae. This species is identified as *Neodilsea yendoana* based on morphological and molecular data. This is the first record of the genus *Neodilsea* and *N. yendoana* in Korea.

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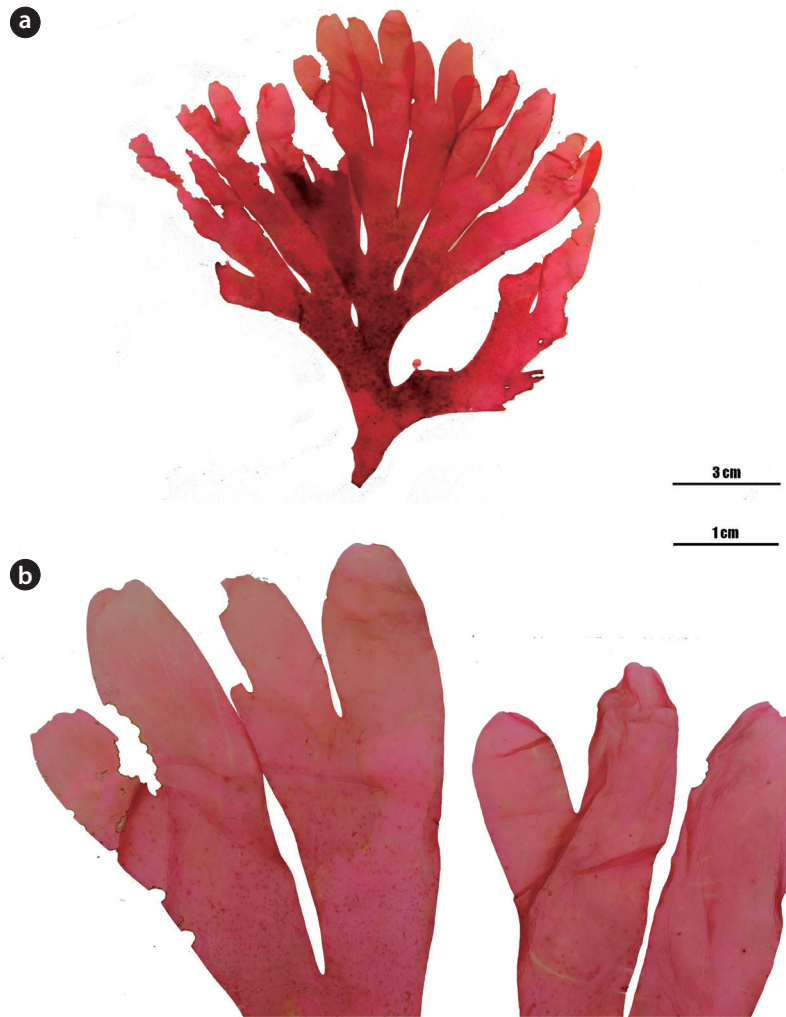


Fig. 1. *Neodilsea yendoana* Tokida. (a) Habit of female plant collected from Jindo, Korea, (b) details of female branches with cystocarps. Scale bars are presented on the figures.

MATERIALS AND METHODS

Specimens for this study were collected along the southern coast of Korea. Taxonomic data were obtained from fresh, liquid-preserved and herbarium specimens. Liquid-preserved material was stored in a 10% solution of formalin/seawater. For anatomical observations, the material was cleared in 5–10% NaOH in distilled water for 2–7 days, and then rinsed in distilled water. Blades dissected from the cleared materials were hand sectioned, transferred to a slide with a drop of distilled water, and mounted in pure glycerin. In some instances, a smearing method for microscopic examination was employed. Measurements are given as widths and lengths. For photographs, the sections were stained with 0.5–1.0% aqueous methylene blue, aniline blue, or hematoxylin. For

permanent slides, the glycerin was exchanged with 10–20% corn syrup.

Total genomic DNA was extracted from silica gel-dried samples 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 by using gel electrophoresis on a 1% agarose gel. Extracted DNA was used for the amplification of ribulose-1, 5-bisphosphate carboxylase large subunit (*rbcL*) regions. PCR amplifications were performed in a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio Inc., Otsu, Japan). The PCR products were moved to the MacroGen Sequencing Service for sequencing (MacroGen, Seoul, Korea). The PCR primers were also used for sequencing.

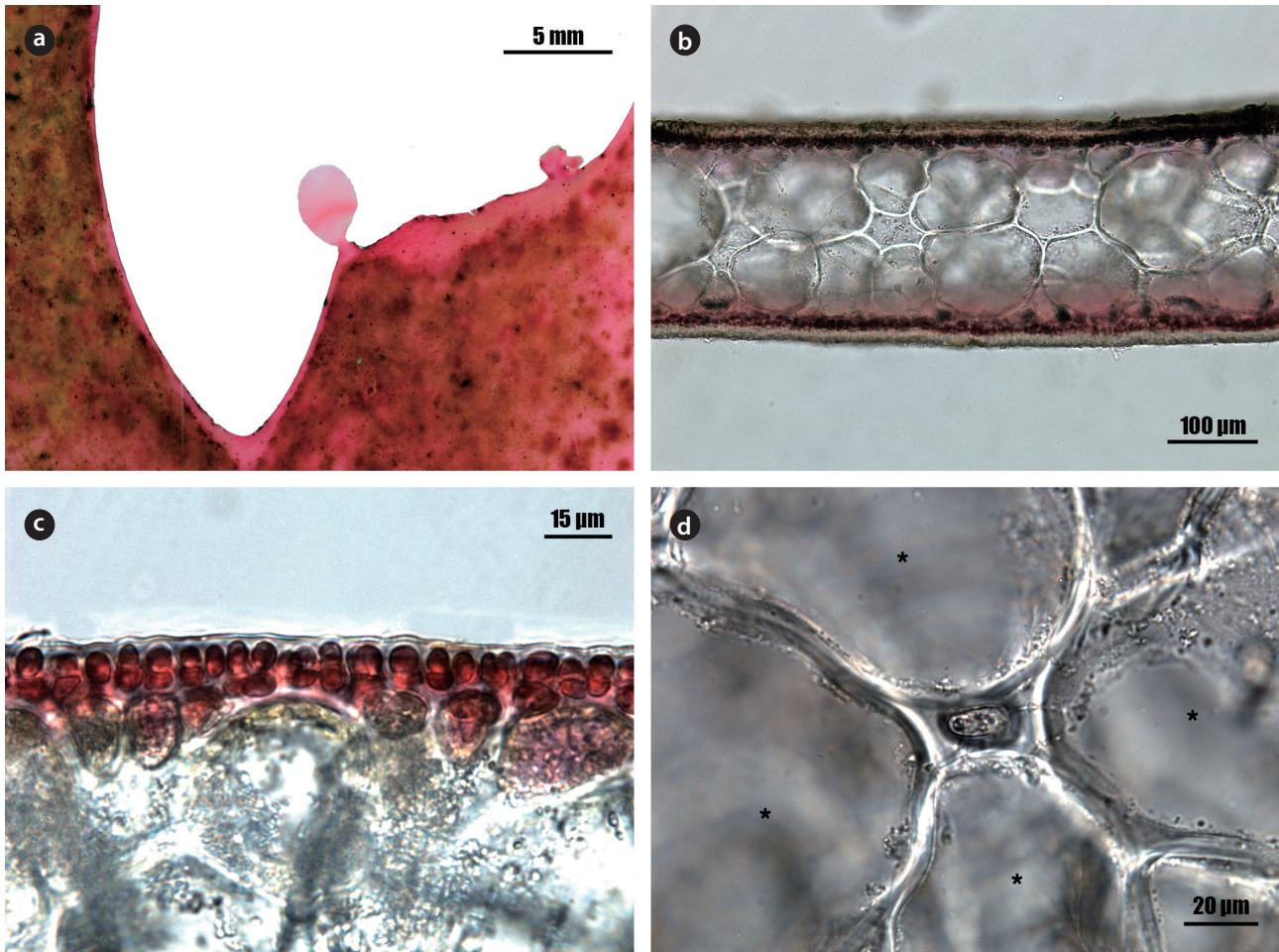


Fig. 2. *Neodilsea yendoana* Tokida. (a) Rarely produced proliferation, (b) large ellipsoid medullary cells in transverse section of branch, (c) cortical cell layers, (d) large axial cells (asterisks) in transverse section of branch. Scale bars are presented on the figures.

Sequences for the *rbcL* region were aligned using BioEdit (Hall 1999). Phylogenetic analyses were performed using the neighbor-joining, maximum-likelihood, and maximum-parsimony methods. Bootstrap values were calculated with 1,000 replications. The *rbcL* sequences of other species were obtained from GenBank. *Dumontia contorta* (S.G.Gmelin) Ruprecht was used as an outgroup.

RESULTS AND DISCUSSION

Neodilsea Tokida 1943: 96

Korean name: Sae-son-ip-sok nom. nov.
(신청: 새손잎속)

Neodilsea yendoana Tokida 1943: 96. figs. 1-9

Korean name: Bulg-eun-sae-son-ip nom. nov.
(신청: 붉은새손잎)

Type locality: Osyoro, Prov. Otaru, Hokkaido, Japan.

Specimens examined: NIBR 0000146289, PKNU 0000 127055, PKNU 0000127048, PKNU 0000127058, PKNU 0000127060 (Jindo: 13.ii.2014).

Habitat: Rock near upper to lower intertidal zone.

Morphology: The thalli are 10–15 cm high (Fig. 1a), flattened, multiaxial, red to brown in color, gelatinous in texture, and attached on the substrate by discoid holdfast. The main axes have dichotomous branches. The branches are divided dichotomously to subdichotomously (Fig. 1b), with a rounded or blunt apex, and an entire margin that is 1–2 cm wide and 300–400 µm thick. The proliferations are rare (Fig. 2a) and arranged pinnately to irregularly. The axial cells are large (Fig. 2d). The cortex consists of small and pigmented cells (Fig. 2b) and is one to two cell layers thick ($4\text{--}5 \times 5\text{--}6 \mu\text{m}$) (Fig. 2c). The medullary cells are arranged in one to three layers and are ellipsoid ($100\text{--}150 \times 50\text{--}100 \mu\text{m}$) in the transverse section.

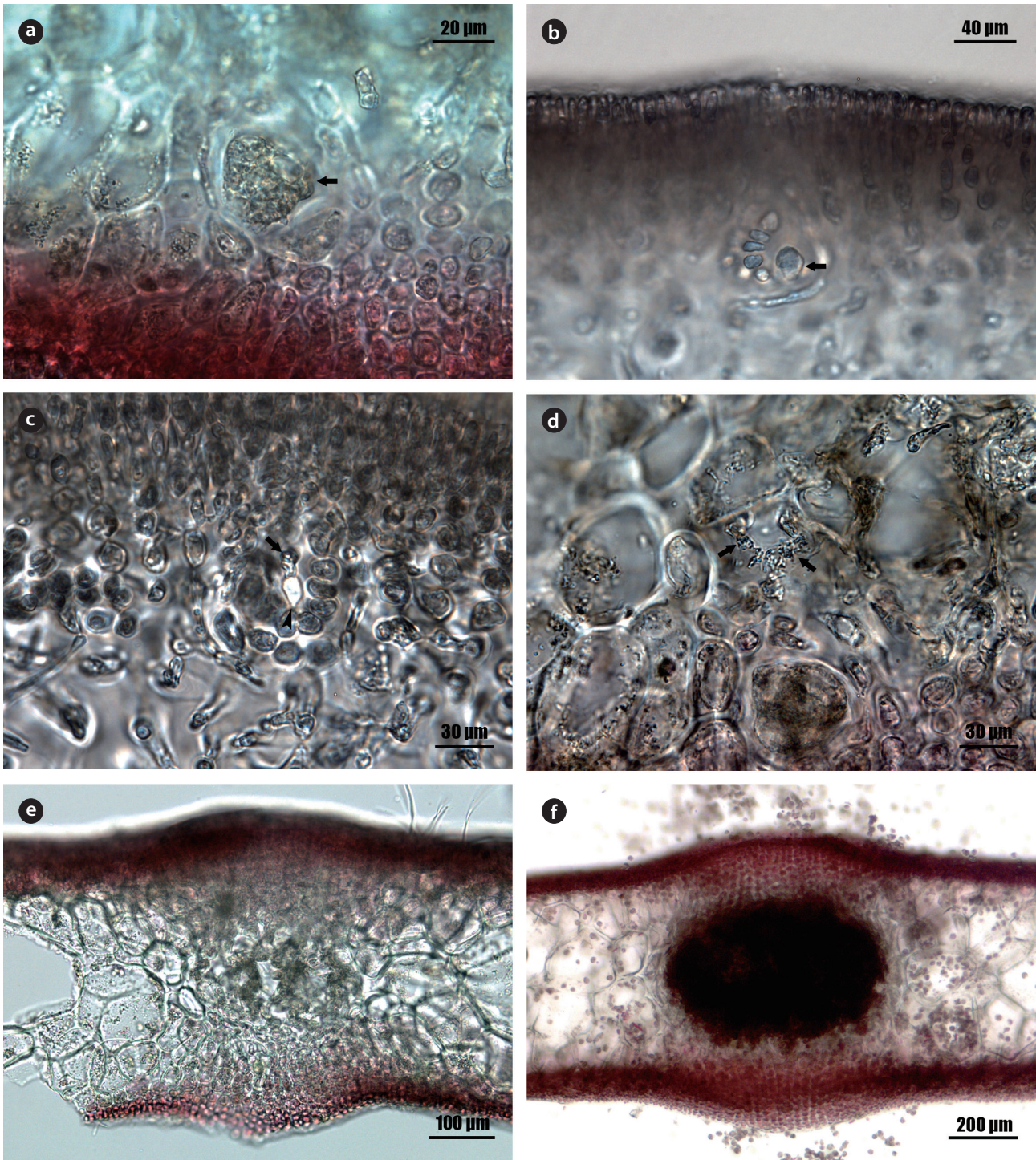


Fig. 3. *Neodilsea yendoana* Tokida. (a) Large supporting cell (arrow), (b) supporting cell (arrow) with carpogonial branch, (c) gonimoblast initial (arrow) produced from auxiliary cell (arrowhead), (d) gonimoblast filaments (arrows) developing inwardly, (e) carposporophyte in early stage, (f) solitary developed cystocarp with protrusion on both surfaces. Scale bars are presented on the figures.

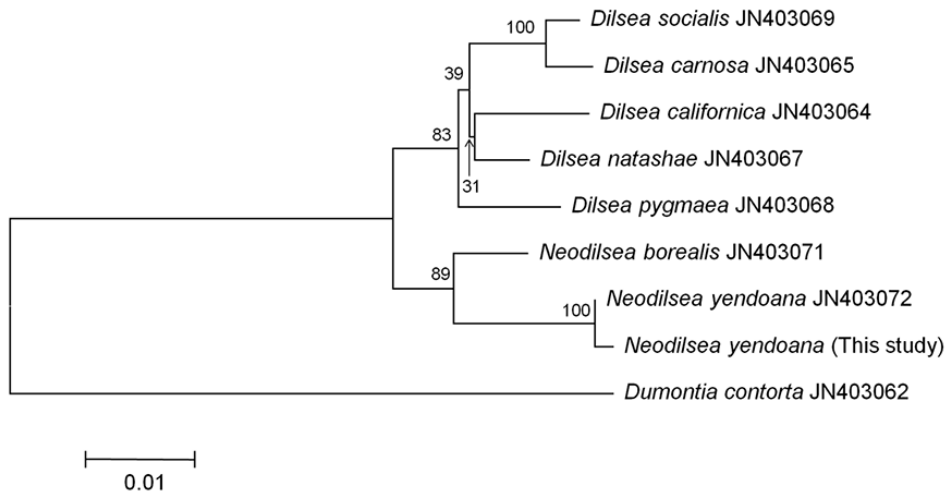


Fig. 4. Phylogenetic tree of *Neodilsea* species obtained from neighbor-joining method based on *rbcl* sequences. Bootstrap proportion values (1,000 replicates samples) are shown above branches. Scale bar, 0.01 substitutions/site.

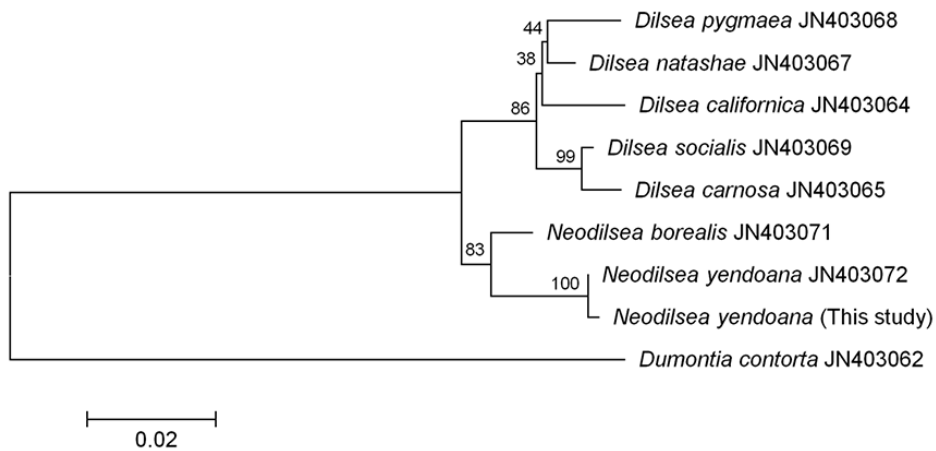


Fig. 5. Phylogenetic tree of *Neodilsea* species obtained from maximum-likelihood method based on *rbcl* sequences. Bootstrap proportion values (1,000 replicates samples) are shown above branches. Scale bar, 0.02 substitutions/site.

In female plant, the supporting cell is intercalarily large and ellipsoidal (Fig. 3a). The carpogonial branch is produced from the large supporting cell (Fig. 3b). The gonimoblast initial is produced from auxiliary cell transformed from supporting cell (Fig. 3c). The gonimoblast filaments develop inwardly toward medulla (Fig. 3d), and then form carposporophytes with masses of carposporangia. The carposporangia are round and 10–12 μm in diameter. The cystocarps are immersed in the medulla solitarily or in irregular groups, but are protruded toward surface. They have a thickened pericarp (Fig. 3e and 3f). Male and tetrasporangial plants were not collected during the present study.

Neodilsea yendoana is morphologically similar to *N. borealis*, *N. crispata*, *N. orientalis*, and *N. tenuipes* in having a dichotomous branching pattern and compressed axes. Of these, *N. borealis* and *N. crispata* are the most similar to *N. yendoana*, in that they have a gelatinous texture and erect and linear branches. However, *N. yendoana* is readily distinguished from the two species, as proliferations are rarely produced near the apex of their branches. In those species, proliferations are pinnately or alternately arranged on the branches. *N. tenuipes* (which shows, together with the dichotomous branching, narrow axes and rare proliferations except for on the apex) differs from *N. yendoana* in thallus size. *N. tenuipes* shows a large thallus

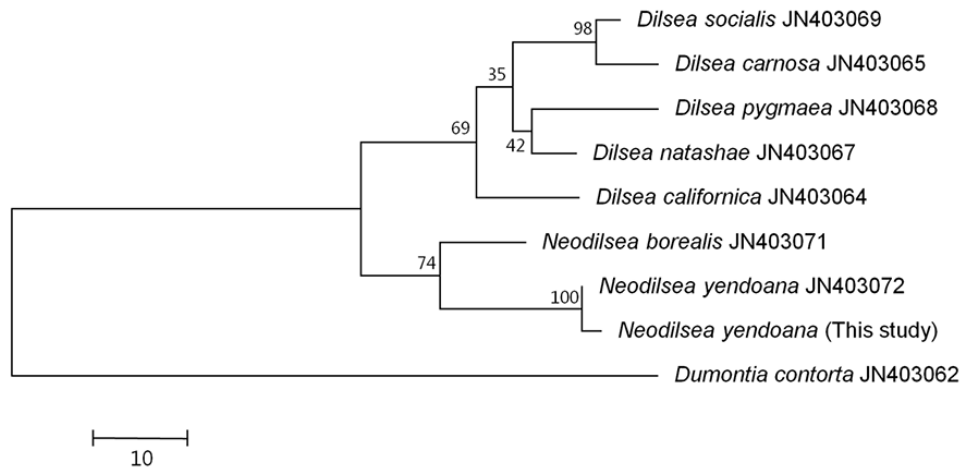


Fig. 6. Phylogenetic tree of *Neodilsea* species obtained from maximum-parsimony method based on *rbcL* sequences. Bootstrap proportion values (1,000 replicates samples) are shown above branches. Scale bar, 10 changes.

(20–30 cm), while that of *N. yendoana* is relatively small (10–15 cm). *Neodilsea orientalis* can be confused with *N. yendoana* in gross morphology. In texture, however, the former species has a membranous thallus, while the latter species has a gelatinous one. *N. yendoana* is characterized by a large and cartilaginous thallus, a dichotomous branching pattern, compressed axes, and rare proliferations near the apex. The Korean alga collected from Jindo in the present study shares these morphological features found in *N. yendoana*.

In a phylogenetic tree based on the *rbcL* sequence (Figs. 4–6), this Korean alga nests in the same clade as *N. yendoana* from Japan. The genetic distance between both sequences within the clade was calculated as 0.2%. Based on the genetic divergence range of 2.0–3.8% found between other *Neodilsea* species (the present study), this distance value is considered to be in the intraspecific range. This Korean alga is identified as *N. yendoana* based on the morphological and molecular analyses. This is the first record of the genus *Neodilsea* and *N. yendoana* in Korea.

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