A Morphological Study of *Dilophus okamurae* (Dictyotales, Phaeophyceae) in Japan

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Sporophytes, male and female gametophytes of *Dilophus okamurae* were collected from Tateyama Bay, Japan. This is the first record of gametophytes for this species. The thicker plants with a multilayered medulla grow mainly in spring to autumn and are all sporophytes. The thinner plants with a unilayered medulla grow in autumn to winter and are either sporophytes or gametophytes. They are similar to *Dictyota* species in morphology. In the thinner plants, sporangia are scattered irregularly on the surface, oogonia are aggregated into rounded sori, and antheridia are aggregated in sori with rectangular outline. This study also proves that *Dilophus okamurae* has different morphological types according to the changes of the environments and the seasons, although sometimes they are sympatrically mixed with each other.

**Key words:** Dictyotales, *Dilophus okamurae*, morphology, Phaeophyceae, reproductive structures.

*Dilophus* was established by Agardh (1882) and was separated from the genus *Dictyota* based on its stoloniferous base and multilayered medulla. Most species of *Dilophus* occur in the southern hemisphere, especially southern Australia (Womersley 1987, Phillips 1992). In Japan, Okamura (1913) described *Dictyota marginata* Okamura, and later he transferred it to the genus *Dilophus* as *Dilophus marginata* (Okamura) Okamura, owing to the peculiar structure of multilayered medulla at the margin (Okamura 1915). Because the name *Dil. marginata* is a later homonym of *Dil. marginatus* J. Agardh, a new name, *Dil. okamurae* was proposed by Dawson (1950).

*Dilophus okamurae* was originally described based on tetrasporophytes occurring in summer (Okamura 1913). Phillips (1992) described the reproductive structures of several species of *Dilophus* from Australia in detail. Hörning et al. (1992) merged *Dilophus* with *Dictyota*, because a multilayered medulla is not clearly differentiated in the north Atlantic species. On the contrary, about the north Pacific species, the molecular data of Dictyotales are not matched with this treatment (Lee and Bae 2002, Hoshina et al. 2004). However, morphological data on the sexual reproductive structures of *Dil. okamurae* have never been reported.

We collected tetrasporophytes as well as male and female gametophytes of *Dilophus okamurae* from the Pacific coast of Japan. The morphology of these reproductive structures is described here in detail. The taxonomic significance of vegetative and reproductive characters and morphological variation of this species are also discussed.
Fig. 1. Collection site of *Dilophus okamurae* in Japan.

**Materials and Methods**

Monthly or seasonal collections were made from April 2003 to May 2004 at Tateyama Bay of the Boso Peninsula, Japan (34°58'N, 139°46'E) (Fig. 1).

The habitat of the species is in the low intertidal and subtidal zone at a depth of 1–5 m. In order to observe the development of reproductive structures, some fresh plants were transported to the laboratory in cooled containers with seawater. For observation of reproductive cells, mature thalli were placed in petri-dishes with sterilized seawater over night, and spores, eggs and antherozoids were released. The other collections were immediately fixed with 10% formalin-seawater soon after collection and preserved for detailed anatomical examination.

Sections for microscopic examination were made from liquid-preserved specimens on freezing microtome (Yamato Koki, Wako). They were stained with 1% aqueous cotton blue solution, and mounted in about 30% corn sugar syrup solution prior to microscope examination. Photographs were taken with light microscope (BH-2 Olympus, CK-2 Olympus) and digital camera (C-3030 Olympus). Voucher herbarium specimens have been lodged in the Laboratory of Phycology, the Tokyo University of Marine Science and Technology (MTUF-AL).

**Results**

*Dilophus okamurae* occurs throughout the year in this habitat, and its morphology varies with the environments and the seasons. Individuals of this population mainly show the two types of thallus morphology, probably due to the seasons: the thinner plants with unilayered medulla occur in lower water temperature (under 20°C) season (Fig. 2), and the thicker plants with multilayered medulla at the margin occur mainly in higher water temperature (20–25°C) season (Fig. 3). Some intermediate types were also collected. Before the thinner plants become dominant some thicker plants tend to be thin (Fig. 4). Early in summer most plants are thin at the upper part and thick at the lower part (Fig. 5). The thicker plants are similar to the plant originally described by Okamura (1913). The gametophytes of the thinner plants were discovered only once in the middle of March 2003, while no gametophytes of the thicker plants were found in the field.

**Morphology of thinner plant**

The thinner plants are always attached to red algae, e. g., *Gelidium* spp. and *Corallina* spp. The narrow thalli are a bit spiral and branched many times, and they have strong
fluorescence in water. Larger individuals attained 15–20 cm in height, internodes are 5–8 mm in length and 2–4 mm in breadth. The blades are about 100–120 μm thick at the center. The upper part of the thalli have a unilayered medulla (Fig. 6). At the lowest part of the blades, the medulla sometimes consists of more than two cell layers (Fig. 7). Reproductive structures are spread on both surfaces of the blades.

1) Tetrasporangia

Initially the darker cortical cells occur randomly on the surface (Fig. 8) and then they swell into spheres protruding from the cortex. Later they become scattered irregularly on the surface (Fig. 9) and sometimes they develop into a group with an obscure outline. Each sporangium is borne on one basal stalk cell, but sometime more than one basal stalk cell can be observed (Fig. 10). Well-developed sporangia are 89.1 ± 9.6 μm in diameter in surface view. Mature sporangia divide tetrahedrally forming four tetraspores (Fig. 11). Released tetraspores are

Figs. 2-5. Plants of *Dilophus okamurae*. Fig. 2. Thinner plant. Fig. 3. Thicker plant. Fig. 4. An intermediate type plant (collected on October 2003). Fig. 5. An intermediate type plant (collected on May 2004).
Figs. 6-7. Sections of a thinner plant. Fig. 6. Upper part, showing unilayered medulla at the margin. Fig. 7. Lowermost part, showing multilayered medulla.

Figs. 8-11. Tetrasporangia of a thinner plant. Fig. 8. Darker cortical cells in cortex. Fig. 9. Tetrasporangial sori irregularly scattered on the surface. Fig. 10. Transverse section of a sporangial sorus, of which sporangia are mostly borne on one basal stalk cell (arrowhead). Fig. 11. Surface view of a mature tetrasporangium divided into four parts (arrowhead).
Figs. 12–15. Oogonia of a thinner plant. Fig. 12. Cluster of darker cortical cells on the surface of a female plant (arrowhead). Fig. 13. Surface view of oogonial sorus. Fig. 14. Surface view of a well-developed oogonial sorus, of which oogonia are tightly aggregated. Fig. 15. Transverse section of an oogonial sorus.

round, 63.6 ± 3.5 μm in diameter. They germinated soon after their release, and become elliptical in form.

2) Oogonia

Darker cortical cells are aggregated in a cluster at first (Fig. 12) and the cluster expand with the number of cells increasing till the oogonial sorus is completed. Oogonial sorus looks like a dot to the naked eye (Fig. 13) and consists of about 10 to 15 oogonia (Fig. 14). Each oogonium is borne on one stalk cell (Fig. 15). Well-developed oogonia are 53.3 ± 5.1 μm in diameter in surface view. Released eggs are spherical, 68.5 ± 1.1 μm in diameter. They do not germinate, and disintegrated after release.

3) Antheridia

Similar in development to oogonial sori, antheridial sori also arise from the clusters of darker cortical cells (Fig. 16), and are scattered on the surface. Antheridial sorus are angular at the surface view, distinguished from tetrasporangial and oogonial sorus easily (Fig. 17). Differing from the development of oogonia, the cortical cells continue to divide into small loculi, which are small squares in surface view (Fig. 18). Each antheridial sorus is tightly surrounded by many sterile cells. Each well-developed antheridium contains about 20 to 30 layers of loculi (Fig. 19), and an antherozoid exists in each locule. Released antherozoids are
round, 5.1 ± 0.7 μm in diameter, with a single observed flagellum.

**Morphology of thicker plant**

The thicker plants occur in large clusters, showing dark-brown color in seawater. Erect thalli are approximately 10–20 cm in height and 5–10 mm in breadth. Up to 10 successive branches occur and many stolons grow from the lower parts. The blades are 120–180 μm at the center. A holdfast is undistinguishable on thalli growing directly from a parental plant. The marginal part consists of multilayered medulla (Fig. 20), and fully multilayered medulla of 4–8-cell layers (Fig. 21) exist in the lowest part of the blade. Only tetrasporangia are found on the both surfaces of the thicker plant, but they tend to spread on one surface. Sometimes they are arranged into irregular patches. Mature sporangia are 130.9 ± 9.2 μm in diameter in surface view (Fig. 22). Well-developed sporangia are divided into four spores, and they are mostly borne on 2–3 basal stalk cells (Fig. 23). Released spores are variable in size, with regular tetraspores and irregular big spores, 70.2 ± 13.2 μm in diameter. If the sporangium does not release spores, it germinates as a small bud (Fig. 22).

The morphological characters of the thin-
Table 1. Morphological characters of *Dilophus okamurae* from the collection site

<table>
<thead>
<tr>
<th>Characters</th>
<th>Thinner plant</th>
<th>Thicker plant</th>
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<tbody>
<tr>
<td>Thallus surface</td>
<td>smooth</td>
<td>rough, complanate</td>
</tr>
<tr>
<td>Thickness of branch (μm)</td>
<td>less than 100</td>
<td>100–200</td>
</tr>
<tr>
<td>Width of branch (mm)</td>
<td>2–4</td>
<td>5–10</td>
</tr>
<tr>
<td>Times of branching</td>
<td>more than 10</td>
<td>less than 10</td>
</tr>
<tr>
<td>Number of sporangial stalk cells</td>
<td>1–2, mostly 1</td>
<td>1–3, mostly 3</td>
</tr>
<tr>
<td>Diameter of mature sporangium (μm)</td>
<td>89.1 ± 9.6</td>
<td>130.9 ± 9.2</td>
</tr>
<tr>
<td>Diameter of spore (μm)</td>
<td>63.6 ± 3.5</td>
<td>70.2 ± 13.2</td>
</tr>
<tr>
<td>Diameter of oogonium (μm)</td>
<td>53.3 ± 5.1</td>
<td>—</td>
</tr>
<tr>
<td>Diameter of egg (μm)</td>
<td>68.5 ± 1.1</td>
<td>—</td>
</tr>
<tr>
<td>Diameter of antherozoid (μm)</td>
<td>5.1 ± 0.7</td>
<td>—</td>
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Figs. 20–23. Morphology of a thicker plant. Fig. 20. Upper part, showing multilayered medulla at the margin. Fig. 21. Lowermost part, showing fully multilayered medulla. Fig. 22. Surface view of scattered tetrasporangial sori with small buds (arrowheads). Fig. 23. Transverse section of sporangia, showing four-divided sporangia with several stalk cells (arrowheads).
ner plant and thicker plant are summarized in Table 1.

Discussion

Although the thicker plants and the thinner plants are distinguished from each other by the vegetative characters, we can confirm that the two types are the same species, because many intermediate types can be collected.

The thinner plant might have been mis-identified as *Dictyota* species, for the “thin thallus” is always regarded as a normal character of *Dictyota*. On the other hand, the thicker plant is distinct, which had been regarded as a standard *Dilophus* species in its morphology. Phillips (1992) did not describe the different morphological types of *Dilophus*. Morphological differences due to the seasons in Australian species are possibly not so distinct as in *Dil. okamurae*.

Reproductive structures of *Dilophus* project above the thallus surface, that is similar to the other genera of Dictyotaceae. In some species of Dictyotales the reproductive structures are embedded in the thallus, such as *Lobospira bicuspidata* Areschoug (Womersley 1987), *Spatoglossum crasssum* Tanaka (Tanaka 1991), *Dictyopteris muelleri* (Sonder) Reinbold (Phillips 1998) and so on. There is no doubt that *Dilophus* is a member of the Dictyotaceae. However, *Dilophus* was merged into *Dictyota* (Hörning et al. 1992), and the new generic concept was accepted by some algologists (Silva et al. 1996, Littler and Littler 1997, De Clerck and Coppejans 1999, 2003). We attempt to emphasise the presence of the multilayered medulla as the taxonomic character distinguishing these two genera.

Although the multilayered medulla is a variable character with the environments and the seasons, that of the thicker plant can be taken as a very useful taxonomic character to distinguish it from *Dictyota* species. The medullary cells of *Dilophus okamurae* are more than four layers thick, sometimes they reach eight layers at the basal part. The medulla of *Dictyota* species is less than two layers even at the lowest part. A hypothesis can be proposed that a multilayered medulla is a primitive character, and some *Dilophus*-like species lost this primitive character and evolved into *Dictyota* species. The genus *Dilophus* is important in making clear the relationships between several genera in Dictyotaceae. A multilayered medulla is also found at the basal part of some *Pachydicton* species such as *P. coriaceum* (Holmes) Okamura (Hwang et al. 2004), that suggests the common character between *Pachydictyon* and *Dilophus*. Hwang et al. (2004) suggested that *Pachydictyon* should be transferred to *Dictyota*, but they did not think the genus *Dilophus* can be merged into *Dictyota*. Furthermore, from the molecular characteristics of the north Pacific species, *Dictyota* is more closely related to *Pachydictyon* than *Dilophus* (Lee and Bae 2002, Hoshina et al. 2004, Hwang et al. 2004). The above hypothesis may explain the genealogical relation between *Dilophus*, *Dictyota* and *Pachydictyon*. However, we never exclude the possibility that the unilayered medulla evolved from multilayered.

In the thinner and the thicker plants, sporangia are scattered on the surface. Phillips (1992) reported that sporangial sori of *Dilophus intermedius* (Zanardini) Allender & Kraft are formed around hair tufts. The arrangement of sporangia of *Dil. okamurae* shows a common character to all species of *Dilophus*, and hair tufts are not around sporangia, but hair tufts around sporangial sori exist in many *Dictyota* species. In some *Dilophus* species, such as *Dil. marginatus* and *Dil. robustus* (J. Agardh) Womersley, sporangia are confined to the concavities in the thallus (Phillips 1992), however the thallus of *Dil. okamurae* is complanate, and sporangia tend to be scattered. The gametophytes of *Dil. okamurae* were discovered...
upon examination of the thinner plants. Oogonia are arranged in sori as is typical of Dictyotaceae. However, scattered oogonia of *Dil. marginatus* from Australia have been reported (Phillips 1992). Antheridia of the thinner plant of *Dil. okamurae* are arranged in regular small sori, while antheridia of *Dil. marginatus* sometimes show scattered large sori. *Dilophus okamurae* have 2–3 stalk cells beneath a divided sporangium, one stalk cell beneath an oogonium and antheridium. Phillips (1992) used the number of stalk cells beneath divided sporangia to identify some Australian species. This suggests that the number of stalk cells is another taxonomic character, especially in the thicker plant of *Dil. okamurae*.

In nature, gametophytes were only found in March whereas sporophytes occur all around the year. That is a reason the sexual reproductive plants had not been discovered. Sexual reproductive plants are rarely found in nature among Dictyotales species, and possible reasons were discussed by Mathieson (1966), Barilotti (1971), Liddle (1971) and Allender (1977). In this investigation, thinner tetrasporophytes became abundant from November. Because there were no mature gametophytes before then, it is possible that the thinner sporophytes developed from thicker individuals by vegetative propagation instead of egg germination. We presume that the thinner plants are ephemeral, and their function is to reproduce in a short period during the year, especially gametophyte plants. The thinner plants mainly occur during the lowest water temperature season at the collection site, when the temperature of seawater is below 15°C. De Wreede (1976) suggested that low water temperature is favorable for embryo growth in brown algae. Kain (1989) and Kain and Bates (1993) pointed out that algae anticipate favorable seasons or respond to them. In the area we investigated, this species may use this reproductive strategy that reproductive gametophytes occur in the low water temperature season and fertilized eggs are able to grow well in the most favorable season. On the other hand, because the thicker plants are sporophytic and perennial, the populations are maintained by vegetative propagation of stolons in higher water temperature season.

With respect to other species of Dictyotales, Tanaka (1998) reported that each species has different seasonal morphologies, describing the spring plants of *Dictyopteris undulata* Holmes with wide blades and the autumn plants with slender and reproductive blades. Hwang et al. (2004) reported *Pachydictyon coriaceum* has two seasonal types: the thin cortical-layered plants and the thick cortical-layered ones. *Dilophus okamurae* also shows several morphological types corresponding with variable environmental conditions. It is rather puzzling that different morphologies of one species can be found in the same area at the same time.

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