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Taxonomic reappraisal of *Dilophus okamurae* (Dictyotales, Phaeophyta) from the western Pacific Ocean

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Dilophus okamurae, a common species of brown alga in the warm temperate western Pacific Ocean (Philippines, Taiwan, China, Korea and Japan), is characterized by a typical vegetative morphology that would support its traditional placement in the genus *Dilophus*. The species has a unilayered cortex, and the medulla is multilayered at least near the margins of the thallus. Sporangia on a two-celled stalk, without an involucrum, however, indicate a close relationship with the recently described genus *Rugulopteryx*. Molecular phylogenetic analyses, based on three separate loci, large subunit rDNA (LSU), *rbc*L and *psb*A confirm the close phylogenetic relationship between *D. okamurae* and *R. radicans*, and *R. marginatus* and *R. suhrii*. A transfer of *D. okamurae* to the genus *Rugulopteryx* is therefore proposed. Contrary to the expected diplobiontic life cycle, typically encountered in Dictyotales, the species exhibits an asexual life cycle consisting of sporophytes only in the populations studied on the east and south coast of Korea. Gametophytes were only obtained by culturing field collected specimens.

KEY WORDS: *Dictyota*, *Dilophus*, *Rugulopteryx*, *Dilophus okamurae*, Life cycle, LSU rDNA, Phylogeny, *psb*A, *rbc*L

INTRODUCTION

The family Dictyotaceae is subdivided into the two tribes Dictyoteae and Zonarieae on the basis of the number of meristematic cells at the frond apices. The Zonarieae have a row or a small group of such cells. In contrast, members of the Dictyoteae are characterized by a single, transversely oriented, lenticular apical cell (De Clerck *et al.* 2006). Generic delineation posed relatively few problems in the Zonarieae, but the Dictyoteae has had a long and troublesome taxonomic history. The distinction of *Dilophus* from *Dictyota* has been particularly problematic. *Dilophus* was erected by J. Agardh (1882), who included six species, *D. repens* (J. Agardh) J. Agardh, *D. gunnianus* J. Agardh, *D. fastigiatus* (Sonder) J. Agardh, *D. opacus* J. Agardh and *D. alternans* J. Agardh. A few years later, De Toni (1891) designated *D. gunnianus* as generitype. The grounds on which *Dilophus* was segregated from *Dictyota*, i.e. the presence of a multilayered medulla, however, was questioned by Setchell and Gardner (1925). Dawson (1950), studying Pacific Mexican species, reported that many species, traditionally assigned to the genus *Dictyota*, possessed a multilayered medulla and/or cortex at least in some part of the thallus. Dawson's findings indicated that generic delineations of *Dictyota, Dilophus* and *Pachydictyon*, and by extension in the entire Dictyoteae the tribe to which they were assigned, were problematic. Despite this criticism, no one attempted a merger of the genera of the Dictyoteae until Hörnig *et al.* (1992a, b) demonstrated experimentally in culture that the number of medullary cells could be altered in many species depending on the culture conditions. Although several authors followed Hörnig *et al.* (1992a, b) in their decision to merge *Dilophus* into *Dictyota*, this taxonomic recommendation was not accepted unanimously, and a minority of phycologists continued to recognize *Dilophus* as a separate genus. A recent study based on ribosomal and plastid sequences has altered our understanding of the generic relationships within the Dictyoteae considerably. De Clerck *et al.* (2006) demonstrated that *Glossophora*, *Glossophorella* and *Pachydictyon* were resolved within a large genus *Dictyota*. Hence, a merger of these genera in *Dictyota* was proposed. Simultaneously two new genera, *Canistrocarpus* (incorporating *Dictyota cervicornis* Kützing, *D. crispata* Lamouroux and *D. magneana* De Clerck & Coppejans) and *Rugulopteryx* [accommodating *D. radicans* Harvey, *Dilophus suhrii* (Kützing) Papenfuss and *D. marginatus* J. Agardh] were proposed. Unfortunately no decision could be made regarding the status of the genus *Dilophus* because of the absence of the generitype, *D. gunnianus*, from the analyses. Therefore, only those species of *Dilophus* included in the molecular analyses were transferred to either *Dictyota* or *Rugulopteryx*. The status of several other species could not be verified then because of the absence of critical morphological data as well as DNA sequences. In this study we review the taxonomic status of *Dilophus okamurae* based on morphological characteristics of vegetative and reproductive structures and interpret the molecular phylogeny based on *rbc*L, *psb*A and LSU gene sequences.

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MATERIAL AND METHODS

Fieldwork and morphological analyses

Specimens of *D. okamurae* were collected in 1994 from 28 populations along the east and south coasts of Korea including Jejudo and Ulreungdo Island. Phenological data were derived from a population at Gangnung, which was monitored monthly during an entire year from January to December in 1994. These phenological data were supplemented with observations derived from four additional populations (Wando, Dolsando, Guryoungpo and Jejudo) that were only occasionally monitored. Plants for morphological analyses were collected in August 1994 and preserved in 5–10% formaldehyde–seawater. Live plants were brought to the laboratory for initiation of unialgal cultures and DNA extraction. The length and width of medullary and cortical cells were measured in surface view or longitudinal section. Thickness of the axes and the height of the respective cells were measured in transverse section. Reproductive structures were measured in surface view as well as transverse section. Sections were made by a Leica Cryocut 1800 microtome^{TM} (Nassloch, Germany), stained with 1% aqueous aniline blue solution, and mounted in 1–5% clear corn sugar syrup prior to microscopic examination. Drawings were made using a camera lucida (Nikon 231412^{TM}, Japan). Voucher specimens are preserved in the herbarium of Kangnung National University.

Unialgal cultures

Source material for unialgal cultures was collected in July from six populations (Gangnung, Haegeumgang, Dolsando, Wando, Jindo and Seongsan). Cultures were initiated from spores released from adult plants that were free from epiphytes. Specimens were maintained in sterilized natural seawater enriched with Provasoli medium (Provasoli 1968). Cultures were kept at 20°C ± 1°C under white fluorescent lights at 50–70 μmol m^{-2} s^{-1} and 14 : 10 L : D photoperiod.

Phylogenetic analyses

Specimens used for molecular phylogenetic analyses are listed in Table 1. DNA extraction, amplification and sequencing of the *rbc*L gene and partial large subunit of the nuclear encoded rDNA (LSU) follow De Clerck *et al.* (2006). The plastid encoded *psb*A gene was amplified using primers listed by Yoon *et al.* (2002). The *rbc*L and *psb*A sequences were aligned by eye using BioEdit 7.0.4.1 (Hall 1999); the LSU dataset was aligned on the basis of secondary structure information with DCSE v. 2.60 (De Rijk & De Wachter 1993; see De Clerck *et al.* 2006 for details). Sequences were analyzed separately as well as combined using a likelihood approach. Maximum likelihood (ML) analyses were carried out in PAUP 4.0b10 (Swofford 2002). MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) was used for Bayesian phylogenetic inference (BI). The best-fitting base-substitution models and corresponding settings for ML analysis were determined using Modeltest 3.7 on the basis of the Akaike Information Criterion (Posada & Crandall 1998). The following settings were used for the ML analyses: starting trees obtained by stepwise addition, random sequence addition (250 replicates), TBR branch swapping and MULTREES in effect. The number of rearrangements was limited to 10,000 per addition–sequence replicate or one hour. Bootstrap analyses consisted of 100 replications with the number of rearrangements limited to 10,000 (or one hour) for each replicate under ML using a neighbor joining tree as starting tree. All Bayesian analyses used a general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR + I + Γ model). Protein coding sequences (*rbc*L, *psb*A) were divided into two partitions corresponding to the first plus second codon position and the third codon position (AAB configuration). A separate GTR + I + Γ model was applied to each partition. For the combined analysis the data set was partitioned according to the different genes, allowing all partitions to evolve at a different rate (ratepr = variable). *Scoresbyella profunda*, the sister taxon of the Dictyoteae (De Clerck *et al.* 2006) was used as the outgroup in all analyses. All analyses were run for one million generations, with two parallel runs of four chains each, trees and parameters were sampled every 100th generation. The first 25% of all trees were discarded as burnin. Consensus trees illustrating incongruence between different analyses methods were constructed using Z-closure networks implemented in Splitstree 4 (Huson & Bryant 2006; see also Holland *et al.* 2005).

RESULTS

Habitat and phenology

Dilophus okamurae is a common species along most of the Korean coast, except for the west coast where it appears to be absent. The alga grows in the shallow subtidal, 0.5–5 m deep, occasionally down to 15 m. At Gangnung the species is present year-round, but is most abundant during summer (Figs 1–12). During winter, plants are reduced to a perennial stoloniferous basal system. From early May onwards many germlings are apparent in the populations, and young shoots develop from the perennial bases (Figs 1–4). Mature sporophytes were observed from June to October, with their highest abundance in September when 20–55% of all individuals in a population were fertile. Despite the abundance of fertile sporophytes no gametophytes were observed during the present study. Plants, therefore, were assumed to propagate asexually in the study area.

Vegetative morphology

Dilophus okamurae is characterized by a dichotomously branched thallus, to 15 cm high. The second internode below the apex measures 5.7–6.3 mm distally and 3.5–4 mm proximally. The length–width ratio of internodes varies between 2.4 and 4.1. Two branching patterns can be discerned among mature tetrasporophytes: one is highly branched and the other is relatively sparsely branched. The highly branched specimens are distributed along the south coast and are characterized by internodes, which are more

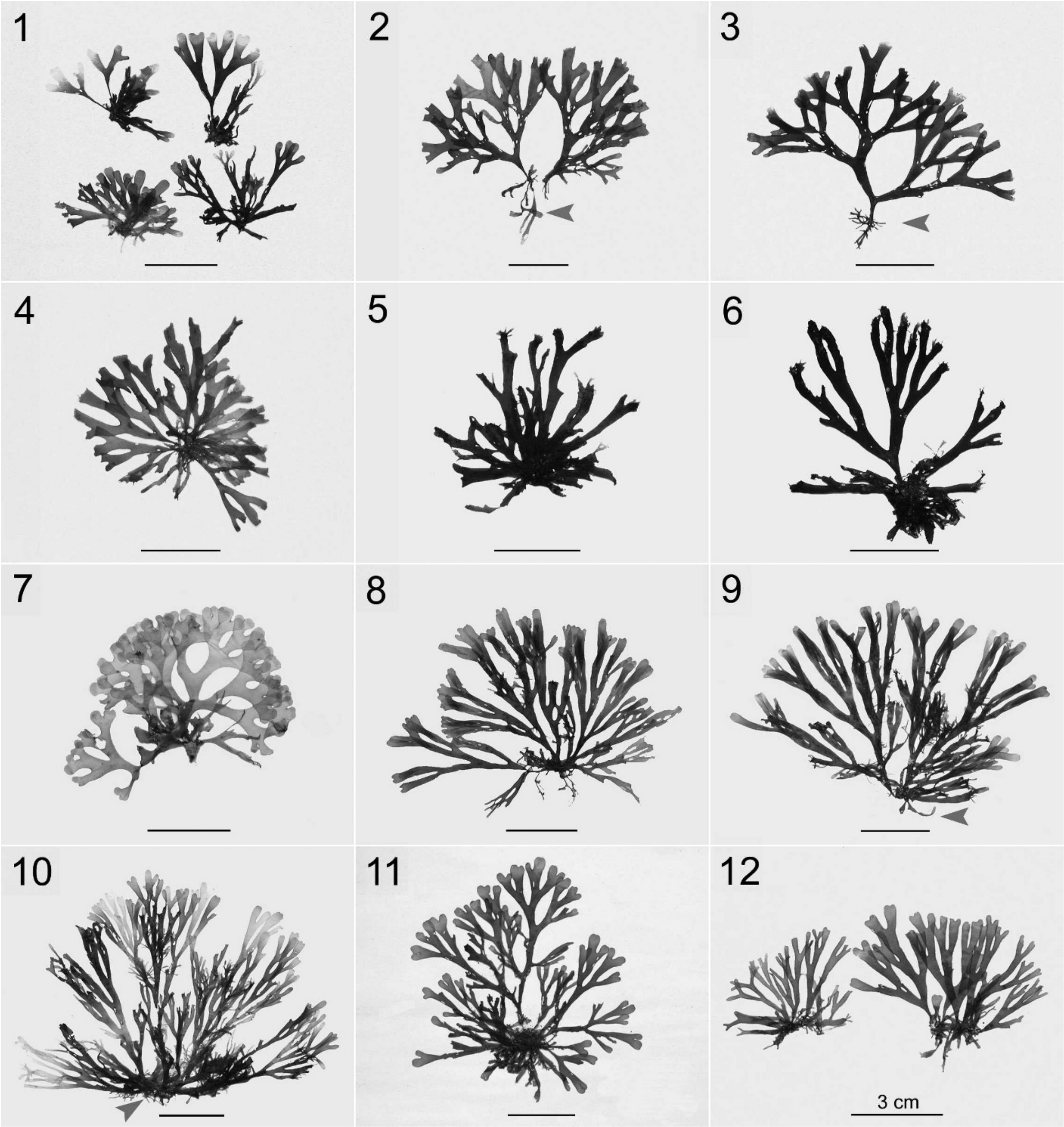
Table 1. Taxa used in this study in the molecular phylogenetic analyses.

Taxa	Collecting data	Accession number		
		*rbc*L	LSU	*psb*A
Canistrocarpus cervicornis (Kützing) De Paula & De Clerck	SE of Olango Island, Philippines (H. Verbruggen, 25 Jan. 2004, HV631)	DQ472048	DQ472103	EU395603
C. crispatus (Lamouroux) De Paula & De Clerck	Hinakpan, Manicani, Guiuan, Eastern Samar, Philippines (D.A. Payo, 5 Aug. 2003, DAP048)	DQ472070	DQ472124	EU395605
Dictyota acutiloba J. Agardh	Ala Moana, Honolulu, Oahu, Hawaii, USA (O. De Clerck, 25 Apr. 2003, ODC888)	DQ472056	DQ472111	EU395602
D. ceylanica Kützing	Faaa, Tahiti, French Polynesia (H. Verbruggen, 21 May 2002, HV214a)	DQ472067	DQ472122	EU395607
D. ciliolata Sonder ex Kützing	SE Olango Island, Philippines (H. Verbruggen, 25 Jan. 2004, HV632)	DQ472053	DQ472107	EU395604
D. coriacea (Holmes) I.K. Hwang *et al.*	Dana Point, Orange County, California, USA (S. Murray, 23 Nov. 2004, CSUF003)	DQ472054	DQ472109	EU395619
D. dichotoma (Hudson) Lamouroux	Point du Nid de Corbet, Audresselles, France (O. De Clerck, 16 Oct. 2004, ODC1027)	DQ472051	DQ472105	EU395606
D. fasciola (Roth) Howe	Ile de Friouel, Marseille, France (O. De Clerck, 23 Sep. 2004, ODC1029)	DQ472074	DQ472126	EU395613
D. intermedia Zanardini	Keppel Bay, Yeppoon, Queensland, Australia (T. Cowling, 18 Aug. 2005, TC1)	DQ472086	EU417906	EU395615
D. jamaicensis Taylor	Drax Hall, East of St. Ann's Bay, St. Ann Parish, Jamaica (H. Verbruggen, 15 Aug. 2004, HV926)	DQ472061	DQ472116	EU395608
D. kunthii (C. Agardh) Greville	Pan de Azucar, Chile (S. Faugeron, Aug. 2004, Chile-M1)	DQ472057	DQ472112	EU395618
D. naevosa (Suhr) Montagne	Mission Rocks, Kwazulu-Natal, South Africa (O. De Clerck & F. Leliaert, 13 Jun. 2003, KZN2241)	DQ472084	DQ472108	EU395609
D. pinnatifida Kützing	Priory Bay, St. Ann Parish, Jamaica (H. Verbruggen, 12 Aug. 2004, HV902)	DQ472059	DQ472114	EU395612
D. sandvicensis Sonder ex Kützing	Lanikai, Oahu, Hawaii, USA (O. De Clerck, 25 Apr. 2003, ODC 896)	DQ472063	DQ472118	EU395611
D. spiralis Montagne	Le Troc, Banyuls sur Mer, France (O. De Clerck, 24 May 2005, ODC1035)	DQ472079	EU417907	EU395616
Dilophus fastigiatus Sonder	Woody Island, Esperance Bay, South Australia (N. Goldberg, 3 Apr. 2003, D96)	DQ472068	DQ472123	EU395614
D. okamurae Dawson	Haegeumgang, South Korea (I.K. Hwang, 11 Jul. 1996, D194)	AY422673	EU417908	AY748322
Rugulopteryx radicans Harvey	Figure of Eight Island, Esperance Bay, South Australia (N. Goldberg, 2 Nov. 2002, s.n.)	DQ472045	DQ472100	EU395610
R. suhrii (Kützing) De Clerck & Coppejans	Palm Beach, Kwazulu-Natal, South Africa (O. De Clerck *et al.*, 11 Nov. 2003, KZN-b 2346)	DQ472044	DQ472099	EU395617
Scoresbyella profunda Womersley	Geographe Bay, Western Australia (J.M. Huisman, Oct. 2003)	DQ472046	DQ472101	EU395620

elongated to 14–16 mm long. The sparsely branched thalli are confined to the east coast, and internodes are only 5–10 mm long. From the base of the thallus terete stoloniferous fibres arise (Figs 2–3, 9). Sori of phaeophycean hairs originate from cortical cells and are irregularly distributed on both sides of the thallus, except the apices and margins. The lens-shaped apical cell is protruding, 55 μm high and 85 μm wide (Figs 13–15), and divides transversely to produce a subapical cell, which undergoes two periclinal divisions, resulting in a three-layered thallus. The primary cortical cells of *D. okamurae* produce a unilayered cortex. Medullary cells of the second internode down from the apex are about 106–130 μm high, 64–87 μm long and 53–58 μm wide in the median region of the axes, and 108–113 μm high, 23–25 μm long and 28–33 μm wide near the margins. Primary medullary cells produce a unilayered medulla except for the marginal part of thallus (Figs 16–19), which becomes multilayered due to transverse division of the internal cells (Figs 20, 22, 23). This multilayered medulla renders the margins thicker than the central portion of thallus. Cortical cells are 38–41 μm long, 12–13.5 μm wide and 17–18 μm high. The cortical cells sporadically produce a cortex two to three cells thick by periclinal division (Fig. 21). Thalli collected from August to November display numerous proliferous branchlets on both surfaces. In culture these proliferations grow into plants of the same life stage as the parent thallus. Ontogenetically such proliferations develop through outward development of a group of adjacent vegetative cortical cells (Fig. 24). Further development is identical to what is described for vegetative axes (Figs 25–28). Proliferous branchlets are readily observed on cultured thalli also (Figs 44–49).

Reproductive structures

Sporangia occur on both thallus surfaces, except for the apical and marginal parts (Figs 29, 32). Mature sporangia are subtended by two stalk cells (Fig. 35) and lack an involucrum. They release either monospores or tetraspores. Undivided sporangia, monospores, often tend to develop *in situ* also. Gametophytes were not encountered in the field. Observations on sexual reproductive structures are, there-



Figs 1–12. Phenotypes of *Dilophus okamurae* Dawson from Gangnung, Korea; collected from January to December respectively in 1994. Arrows in 2, 3, 9, 10 indicate terete stoloniferous fibres. Scale bar applies to all figures.

fore, derived exclusively from cultured plants. Female gametophytes are characterized by sori containing from 3 to 35 oogonia each (Figs 30, 33, 36). Oogonia are 63 µm high and 26 µm wide. Immature oogonia lack a stalk cell (Fig. 36). Male gametophytes have sori containing from 30 to 100 antheridia each (Figs 31, 34). Individual antheridia are 70–80 µm high and 18–23 µm wide. Each antheridium is subtended by a single stalk cell (Fig. 37).

Life history

Six strains collected in July from the east and the south coast (Gangnung, Haegeumgang, Dolsando, Wando, Jindo and Seongsan) were maintained as unialgal cultures. All strains exhibited a regular biphasic life cycle with alternating gametophytes and sporophytes. Excised apical portions of adult tetrasporophytes (*c.* 1 cm) developed tetrasporangia after four weeks and eventually released tetraspores and monospores (Fig. 38), and these spores started to germinate after being released (Figs 39–40). Tetraspores germinated to form fusiform germlings with a discoid rhizoid in *c.* seven days (Fig. 41) and linear rhizoids in *c.* 10 days (Fig. 42). Dichotomous branching was achieved in two or three weeks (Fig. 43). In culture monospores and tetraspores released from field collected plants developed into tetrasporophytes and gametophytes respectively. Male and female gametangia developed on gametophytes after nine weeks. The fertilized eggs germinated and grew into mature tetrasporophytes in 10 weeks. It took about 19 weeks to complete a life cycle with alternating sporophytes and gametophytes in culture.

Molecular phylogenetic analyses

Collection information and accession numbers of all taxa used are given in Table 1. The molecular data set consisted of three genes: a partial LSU rDNA alignment comprising 1328 bp of which 132 positions were excluded from the analyses, and two chloroplast protein coding genes *rbc*L and *psb*A, comprising 1182 bp and 909 bp, respectively. Maximum likelihood and Bayesian analyses of a concatenated dataset containing all three markers resulted in identical tree topologies (Fig. 50). The three genera of the Dictyoteae recognized by De Clerck *et al.* (2006) receive maximum support (posterior probabilities = 1.0 and bootstrap support = 100). The intergeneric relationships are also well resolved with posterior probabilities equal or close to 1.0 and bootstrap support higher than 72. *Dilophus okamurae* forms a clade with *R. suhrii* and *R. radicans*. *Dilophus fastigiatus* is resolved as the sister taxon of *Dictyota* as currently delineated. Intrageneric relationships in the latter genus, however, are only poorly resolved. The uncertainty in generic relationships within *Dictyota* is strikingly reflected by means of a consensus network resulting from superimposing the ML trees of each of the gene regions analyzed separately (Fig. 51). When analyzed separately the individual datasets result in the resolution of the same genera but their relative relationships may differ. LSU and *rbc*L trees are identical, but the *psb*A tree differs in the position of *D. fastigiatus*, which occupies a basal position in the Dictyoteae and the sister relationship of *Canistrocarpus* and *Rugulopteryx* (Fig. 52).

DISCUSSION

In the revision of the genus *Dictyota* and related genera *Dilophus*, *Pachydictyon*, *Glossophora* and *Glossophorella* by De Clerck *et al.* (2006), rather than recognizing five distinct genera, the authors opted for a large genus *Dictyota* that incorporated species with unilayered as well as multilayered medullary and cortical layers. Reproductive structures were thought to be more important as diagnostic characters at the generic level. *Dictyota* was characterized by tetrasporangia subtended by a single stalk cell and lacking a distinct involucrum, and antheridia surrounded by unicellular paraphyses. These characters differentiated *Dictyota* from the newly described genera *Canistrocarpus* and *Rugulopteryx*. No final decision was made on the status of *Dilophus*; although, De Clerck *et al.* (2006) hypothesized that *D. gunnianus* the generitype of *Dilophus*, would most likely be resolved within *Dictyota*. In contrast, earlier studies (e.g. Lee & Bae 2002; Hoshina *et al.* 2004) lent support to the traditional distinction between *Dictyota* and *Dilophus*. However, Lee & Bae (2002) and Hoshina *et al.* (2004) included only a single *Dilophus* species, *D. okamurae*, which invariably occupied a sister relationship to a clade comprising *Dictyota* as well as *Pachydictyon* species. Analyses presented in this study based on LSU rDNA, *rbc*L and *psb*A sequences support the divergent nature of *D. okamurae*. A more comprehensive taxon sampling demonstrates a close relationship between this species and *Rugulopteryx suhrii* and *R. radicans*. These relationships are supported by high posterior probabilities and bootstrap support. Several morphological and reproductive characteristics link these three species. Just like *Rugulopteryx* species, sporangia of *D. okamurae* are borne on two stalk cells. Unlike *R. marginata*, *R. radicans* and *R. suhrii*, the thalli of Korean specimens of *D. okamurae* do not appear undulate. They are flat with reproductive structures evenly distributed over the entire thallus surface, rather than being confined to the concavities as in the other *Rugulopteryx* species. Pictures of specimens from Chiba Prefecture, Japan by Hideki Haga (Guiry & Guiry 2007) show an undulate thallus. This difference indicates that the surface morphology of the thallus may be subject to developmental or intraspecific variation. To some extent it questions the utility of this character at the generic level. However, given the typical morphology of the sporangia and the fact that *D. okamurae* seems closely related to the genus *Rugulopteryx* based on three different genes, we conclude that the species should be transferred to genus *Rugulopteryx* and propose the new combination of this species as follows:

Rugulopteryx okamurae* (Dawson) I.K. Hwang, W.J. Lee & H.S. Kim *comb. nov.

BASIONYM: *Dilophus okamurae* Dawson *Bulletin of the Torrey Botanical Club* 77 (2), p. 86 ['*Okamurai*'] (1950).

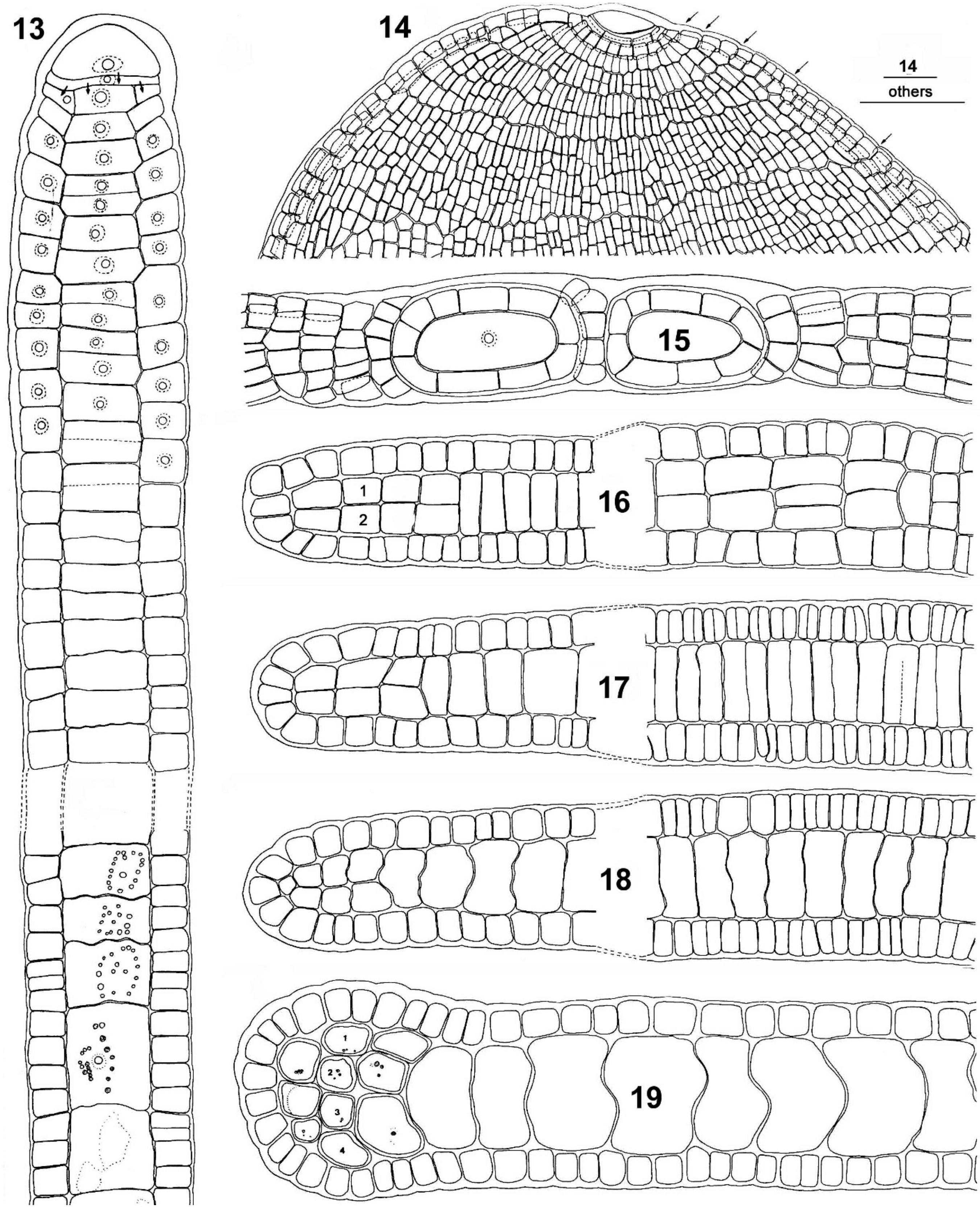
HOMOTYPIC SYNONYMS:

Dictyota marginata Okamura, 1913: 33–35, pl. CVIII: fig. 9; pl. CIX, figs 1–9, *nom. illeg.*

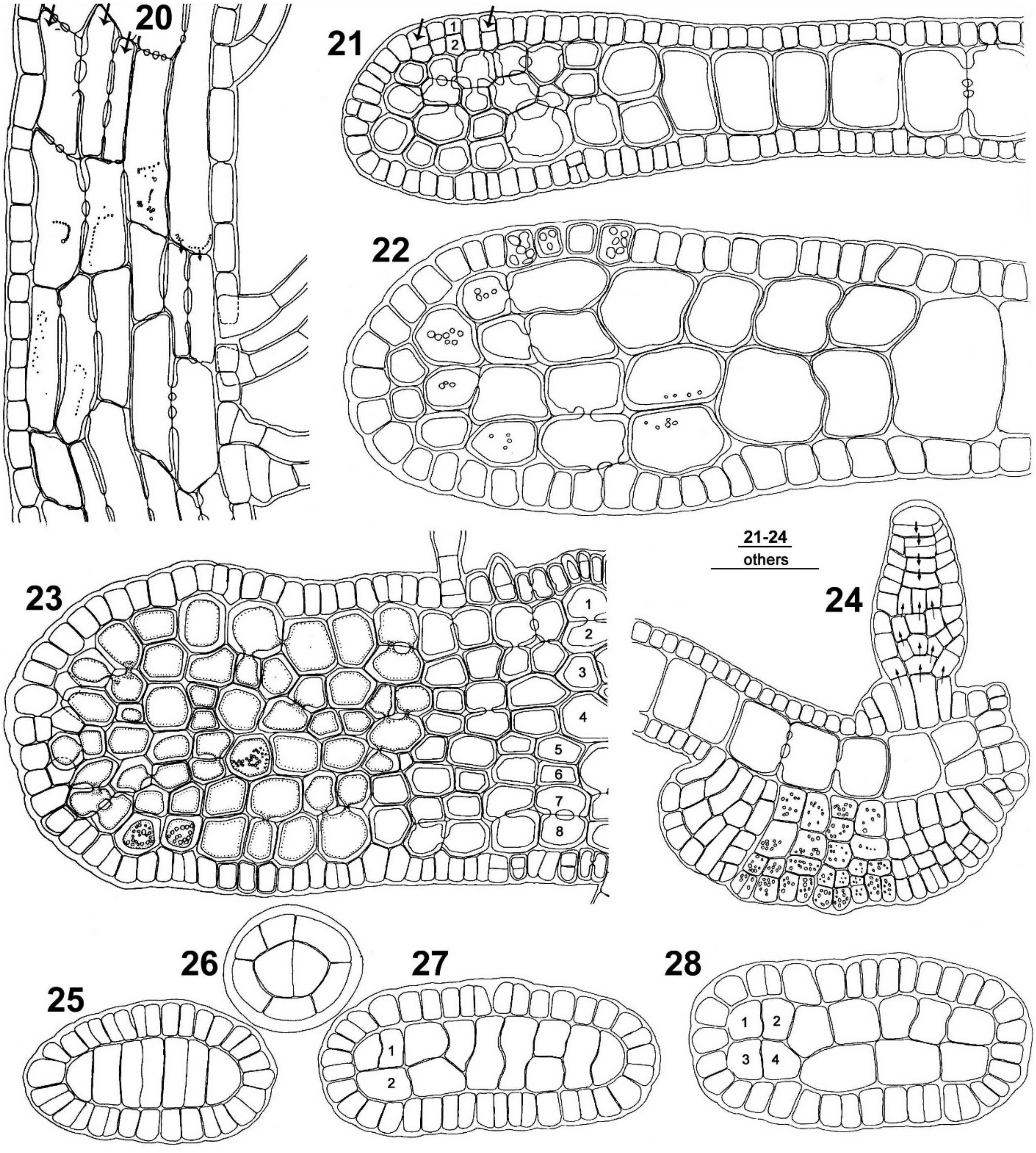
Dilophus marginatus (Okamura) Okamura, 1915, p. 154, *nom. illeg.*

Dictyota okamurae (Dawson) Hörnig, Schnetter & Prud'homme van Reine, 1992a, p. 54.

NOTES: *Dilophus okamurae* Dawson (1950) is a substitute name for *D. marginatus* (Okamura) Okamura (1915). The latter is illegitimate because of the prior existence of *D. marginatus* J. Agardh (1894) [= *Rugulopteryx marginata* (J. Agardh) De Clerck]. Furthermore, *Dictyota marginata* Okamura (1913) is also illegitimate because of the prior existence of *D. marginata* Areschoug (1851) [= *R. suhrii* (Kützing) De Clerck & Coppejans] as well as *D. marginata* (C. Agardh) Greville [= *Stoechospermum polypodioides* Lamouroux]. Dawson's epithet '*okamurae*' is the earliest available one and therefore the basis for the new combination.



Figs 13–19. Anatomical characteristics of *Dilophus okamurae* Dawson from Wando, Korea.
Fig. 13. Longitudinal section of the thallus with apical cell.
Fig. 14. Apex of thallus with single protruding apical cell.
Fig. 15. Transverse section of a thallus near the apex.



Figs 20–28. Anatomical characteristics of *Dilophus okamurae* Dawson from Wando, Korea.
Fig. 20. Longitudinal section of the lower part of thallus showing a multilayered medulla.
Figs 21–22. Transverse section of the second internode down from the apex.
Fig. 23. Transverse section of the lower part of the thallus with a multilayered medulla.
Fig. 24. Transverse section of the thallus with young proliferous branchlet.
Figs 25–28. Transverse section of proliferous branchlets, in upper (Figs 25, 26) and lower parts of the thallus (Figs 27, 28). Scale bars = 50 μm.

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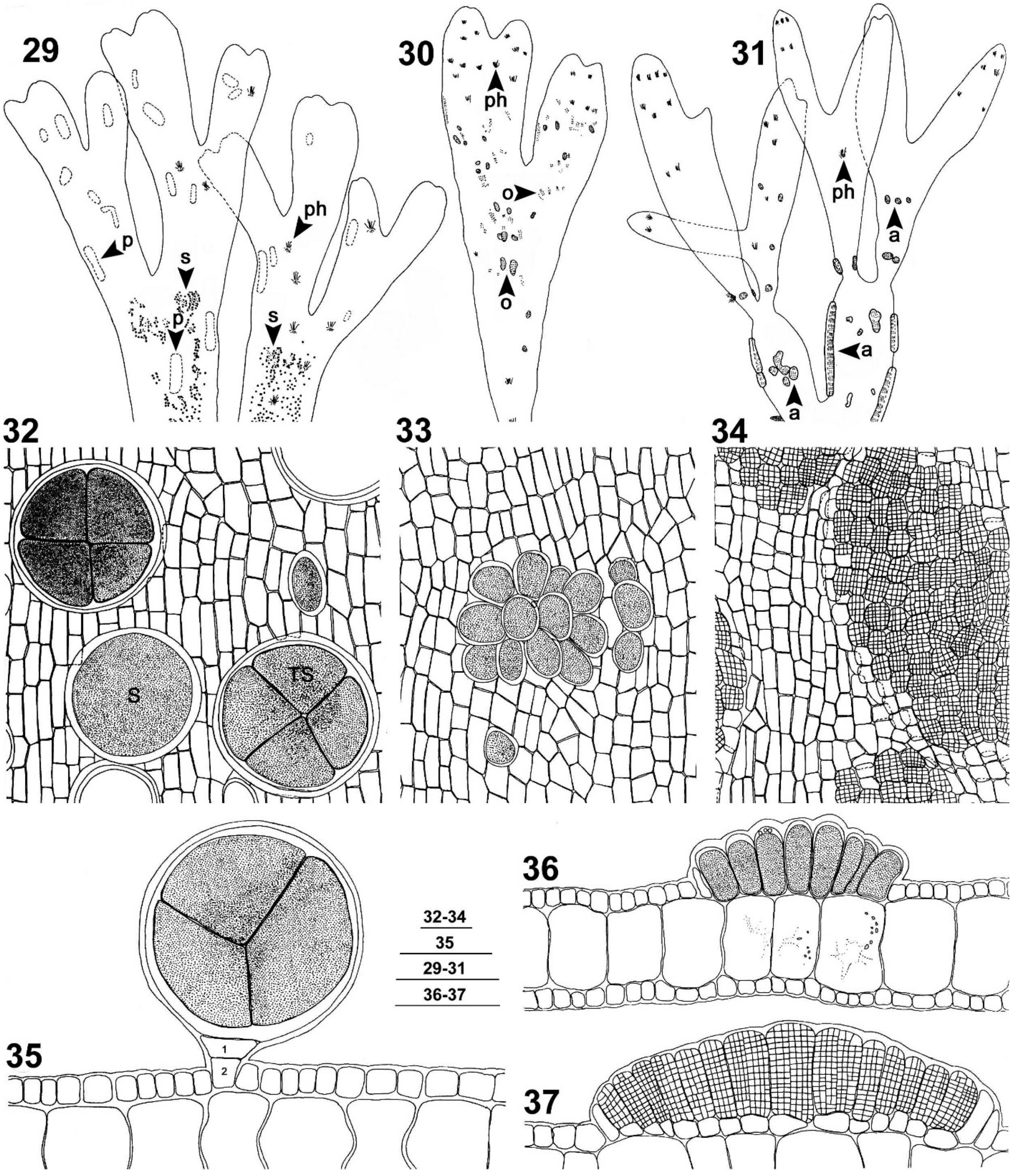
Figs 16–19. Transverse sections of the thallus approximately 40 μm below the apex (Fig. 16); 220–550 μm below the apex (Figs 17–18); and 5 mm below the apex (Fig. 19). Scale bars = 50 μm.



Figs 29–37. Reproductive structures of *Dilophus okamurae* from Wando, Korea (based on field collected tetrasporophytes and cultured gametophytes).

Fig. 29. Distal portion of a thallus showing scattered sporangia.

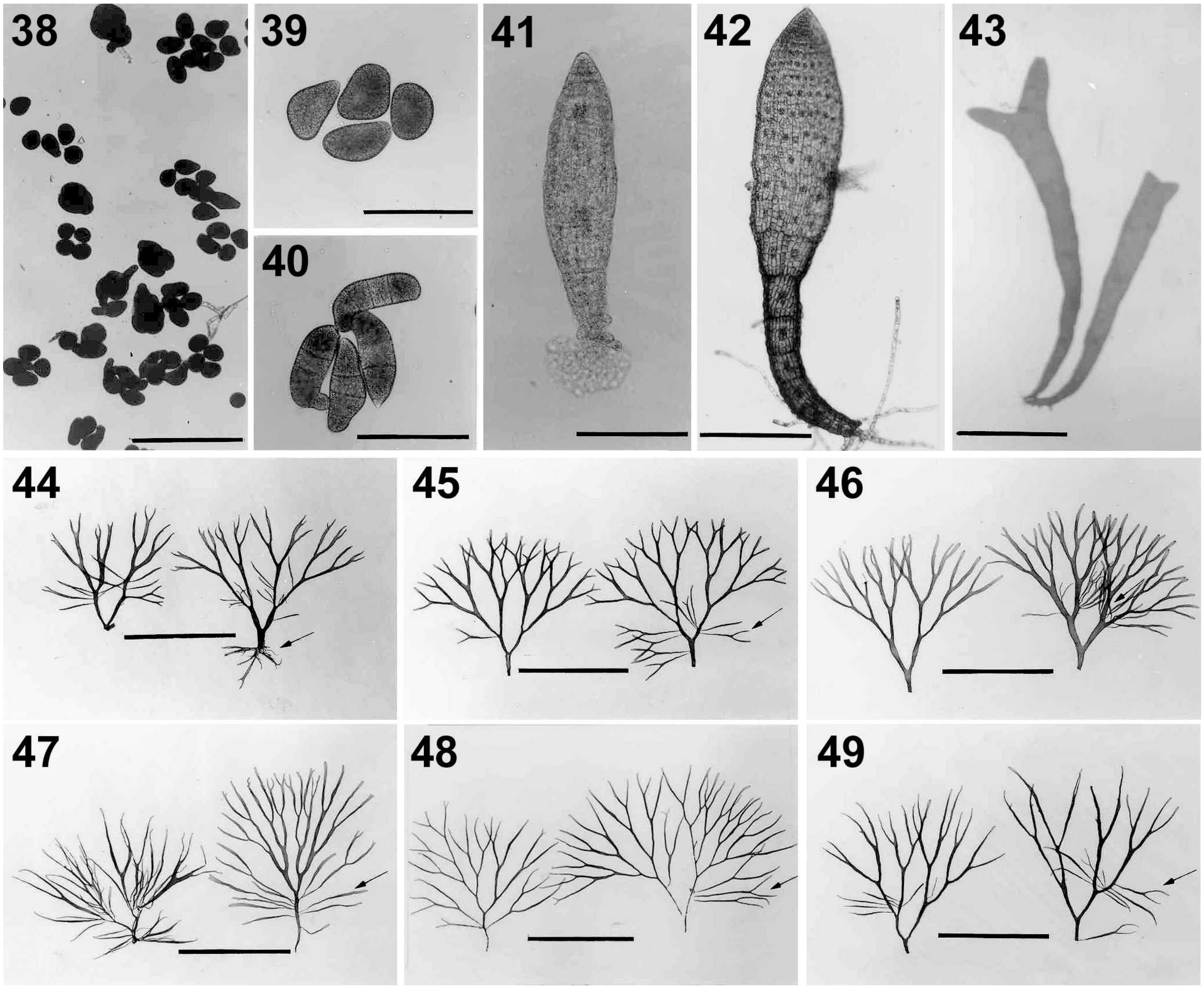
Fig. 30. Oogonial sori.

Fig. 31. Irregularly shaped antheridial sori.

Fig. 32. Surface view of monosporangia and tetrasporangia.

Fig. 33. Surface view of oogonial sori.

Fig. 34. Surface view of antheridial sori.



Figs 38–49. Germination of tetrasporangia and cultured strains *Dilophus okamurae* Dawson from Korea.
Fig. 38. Released tetraspores and monospores from plants from Wando.
Figs 39–40. Germlings of tetraspores in eight hours to one day germination.
Fig. 41. Germling of tetraspores in seven days.
Fig. 42. Young plant in 10 days.
Fig. 43. Young plants with bifurcation in 18 days.
Figs 44–49. All cultured tetrasporic plants from different localities have proliferous branchlets (arrows).
Fig. 44. Gangnung strain.
Fig. 45. Haegeumgang strain.
Fig. 46. Dolsando strain.
Fig. 47. Wando strain.
Fig. 48. Jindo strain.
Fig. 49. Seongsanpo strain. Scale bars = 0.3 cm (Figs 42–43), 3.7 cm (Figs 44–49), 450 μm (Fig. 38), 200 μm (Figs 39–41).

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Fig. 35. Transverse section of a tetrasporangium subtended by two stalk cells.
Fig. 36. Young oogonial sori on cultured plant.
Fig. 37. Antheridial sori on cultured plant. Scale bars = 0.5 cm (Figs 29–31), 50 μm (Figs 32–37).

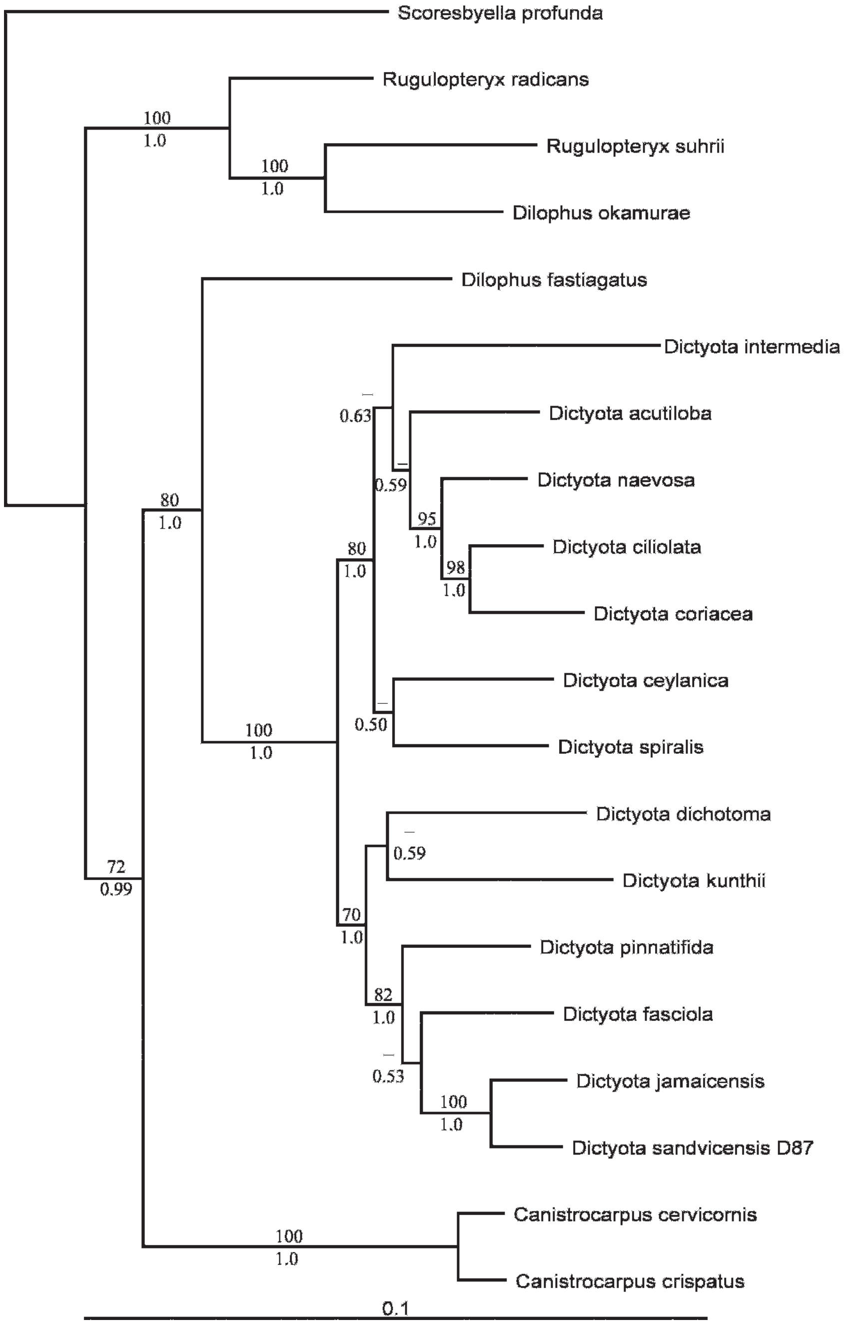

Fig. 50. The consensus tree resulting from the BI analysis combining all three genes. The results of an ML bootstraps analyses are indicated above the branches; posterior probabilities below the branches. Branch lengths are proportional to the number of substitutions per site (see scale bar).

Dilophus okamurae is a common species in the subtropical to temperate western Pacific Ocean (Lee & Kang 1986; Silva *et al.* 1987; Yoshida 1998). Its distribution in Korea is restricted to the east and south coasts as are the distributions of other species of Dictyotales (Hwang *et al.* 2004). *Dilophus okamurae* is abundant year-round although only as dormant rhizoidal bases from January to April. Maximum growth and development of reproductive structures were observed when seawater temperatures exceeded 15°C. This seasonal growth pattern indicates that *D. okamurae* could be considered as a subtropical species that manages to persist in more temperate conditions. Significant in this respect is the failure of *D. okamurae* to complete its sexual life cycle in Korean waters. In the field sporophytes apparently exclusively recycle the diploid life stage and most likely fail to undergo meiosis. No gametophytes were observed. Moreover, *D. okamurae* readily forms propagules, proliferous branchlets arising on the thallus surface which grow into new plants of the same ploidy level. This phenomenon whereby algae reproduce predominantly or exclusively asexually at the high latitude ends of their distribution has been reported in

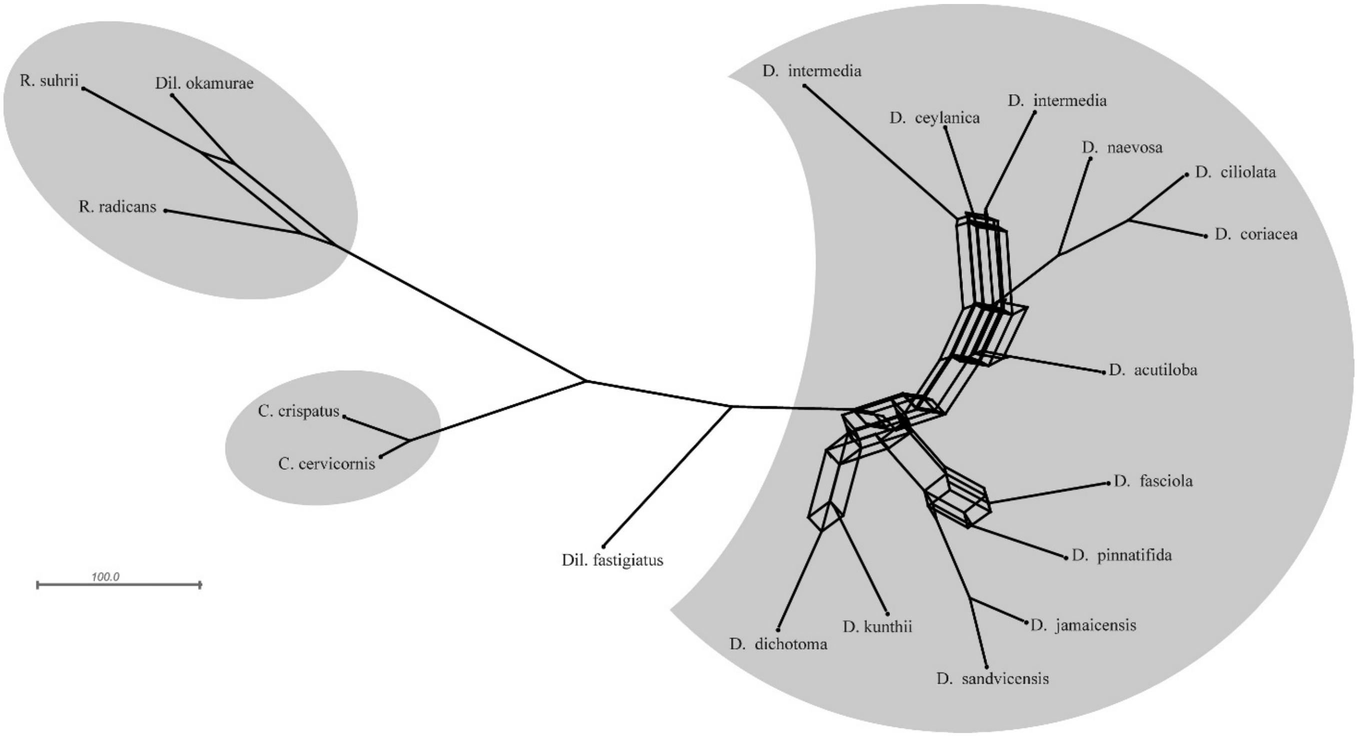

Fig. 51. Consensus network based on the ML trees of each of the three genes analyzed separately. *Scoresbyella profunda*, used as outgroup, was pruned from the respective trees.

several instances (Hawkes 1990; Thornber 2006). Culture experiments whereby *D. okamurae* is maintained at 20°C indicate that environmental conditions, most likely unfavorable sea surface temperatures, cause the alga to reproduce exclusively asexually. Hwang *et al.* (2005) described the same phenomenon for *D. dichotoma* sensu Okamura from Korea.

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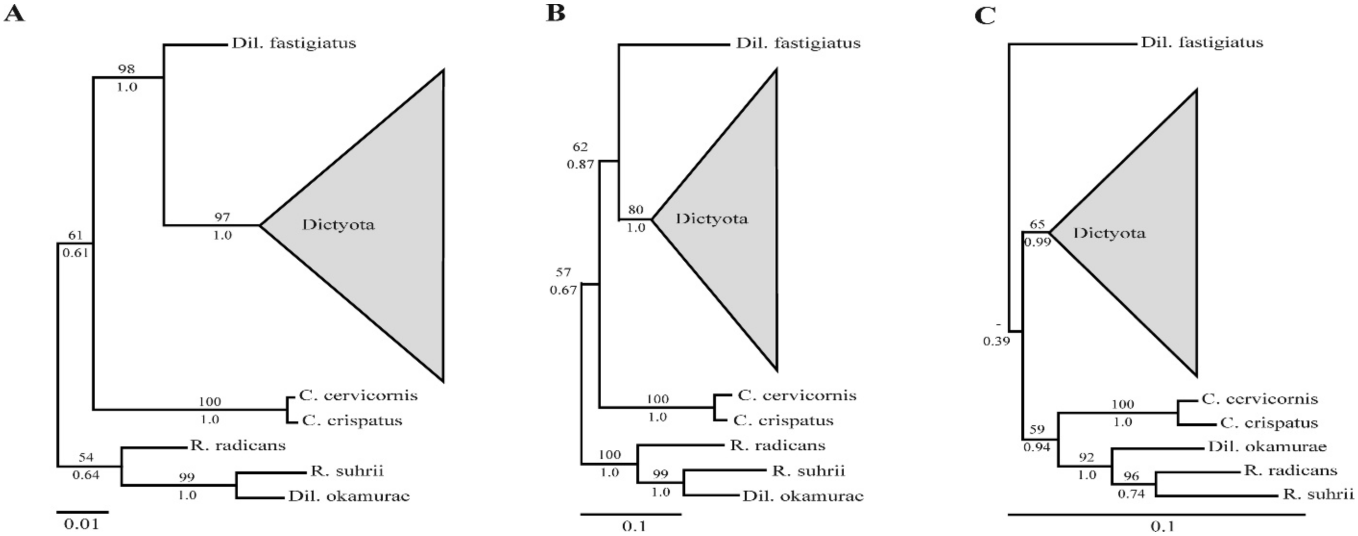

Fig. 52. Generic evolutionary relationships of the Dictyoteae as inferred from ML analyses of the partial LSU gene (A), *rbc*L (B) and *psb*A (C). The results of an ML bootstrap analyses are shown above the branches, Bayesian posterior probabilities are indicated below the branches. *Scoresbyella profunda*, used as outgroup, was pruned from the respective trees. Branch lengths are proportional to the number of substitutions per site (see scale bars).

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