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Generic boundaries and phylogeny of *Campylaephora* (Ceramiaceae, Rhodophyta), including *Campylaephora californica* (Farlow) comb. nov.

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The four described species in the genus *Campylaephora* J. Agardh, tribe Ceramieae, namely, *C. hypnaeoides*, *C. borealis*, *C. crassa* and *C. japonica*, are known only from the western North Pacific. A new combination, *C. californica*, is proposed for plants referred to as *Microcladia californica*, originally described from syntype localities of California and Oregon. The species has alternate branching, six or seven periaxial cells per axial cell, internal rhizoidal filaments derived from periaxial and cortical cells, spermatangia formed from outer cortical cells, tetrasporangia developing from periaxial and inner cortical cells and covered by outer cortical cells, and naked carposporangia without involucral branchlets. *Campylaephora* is redefined to include species characterized by the presence of multiple layers of rhizoidal filaments derived from cortical cells that are linked by secondary pit connections. In our molecular study inferred from plastid-encoded *rbc*L sequence analysis, *C. californica* from San Mateo, California, nested inside the *Campylaephora* clade with strong bootstrap support and was separate from a *Microcladia* species. Our molecular phylogenetic analyses also revealed that *C. californica* is a separate entity among the species of *Campylaephora*. The genus *Campylaephora* forms a terminal clade within the tribe Ceramieae. *Ceramium boydenii* is basal in *rbc*L trees leading to *Campylaephora* and less advanced in possessing single-layered cortical rhizoidal filaments that lack secondary pit connections.

KEY WORDS: *Campylaephora*, *Campylaephora californica*, Ceramiaceae, Phylogeny, *rbc*L, Rhodophyta, Taxonomy

INTRODUCTION

The genus *Campylaephora* was described in 1851 by J. Agardh based on *Ceramium rubrum* var. *firmum* C. Agardh. The thick cortex and hooked terminal portions of branches (J. Agardh 1851) and the rhizoidal cortical filaments (Schmitz & Hauptfleisch 1897) were recognized as key characters of this genus. In 1927, Okamura reduced *Campylaephora* to synonymy with *Ceramium* because he believed that the rhizoidal cells in the cortex were not true rhizoids. Nakamura (1950) resurrected *Campylaephora* after studying its detailed morphology. He was of the opinion that the presence of rhizoidal filaments in the cortex, the conical discoid holdfast, the dichotomous ramification and sickle-shaped portions of the frond, and the large tetrasporangia warranted the recognition of *Campylaephora*. More recently, *Campylaephora* has been regarded as unstable because some of its diagnostic characters overlap with those of *Ceramium* (Simons 1968; Boo & Lee 1994). Thus, there is critical need for a revision of *Campylaephora* and some *Ceramium* species, such as *C. boydenii* E.S. Gepp (Seo *et al.* 2003; Cho *et al.* 2004).

Campylaephora currently contains four species: *C. borealis* (Y. Nakamura) Seo, T.O. Cho & Boo, in which the main branches tend to form percurrent axes that bear proliferous branchlets on all sides; *C. crassa* (Okamura) Nakamura with doubly serrated branchlets on the adaxial sides of branches (Nakamura 1950); *C. hypnaeoides* J. Agardh with portions of the frond sickle-shaped and branches oriented in all directions (Nakamura 1950); and *C. japonica* Noda with small fronds (*c.* 4 mm high) (Noda 1974). All known members of the genus *Campylaephora* are endemics in the northwest Pacific from Korea, Japan, China, and Russia (Okamura 1927; Noda 1974; Boo & Lee 1994; Seo *et al.* 2003).

Microcladia californica Farlow (1875) was originally described from syntype localities of California and Oregon. This species is easily recognized by the presence of rhizoidal inner cortical filaments, a carposporophyte that lacks involucral branchlets, and its especially epiphytic occurrence on the brown alga *Egregia menziesii* (Turner) Areschoug (Abbott & Hollenberg 1976; Gonzalez & Goff 1989a, b). *Microcladia californica* shares some characters with *Campylaephora* from the northwest Pacific, such as internal rhizoidal cortical filaments (Abbott & Hollenberg 1976); however, this species is endemic to California, from San Francisco to San Diego (Abbott & Hollenberg 1976). Recent monographic studies on the morphology, phenology, and culture of *M. californica* were conducted by Gonzalez & Goff (1989a, b, c).

Ceramium boydenii was described by Gepp (1904). The species has been recorded only from the northwest Pacific (e.g. Nakamura 1965; Itono 1977). Nakamura (1954) reported the presence of rhizoidal cortical cells in this species and compared it to species of *Campylaephora*. In a recent paper, Seo *et al.* (2003) suggested that *Ceramium*

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boydenii should be reassessed because it appears to be more closely related to *Campylaephora* than to its congeners. Because *C. boydenii* shares several key characters previously considered to be diagnostic for *Campylaephora* (Nakamura 1954; Boo & Lee 1994; Seo *et al.* 2003), it is important to re-evaluate its taxonomic position and to determine the precise boundary between *Campylaephora* and closely related species of *Ceramium*.

In this study, we investigate three *Campylaephora* species from the northwest Pacific (Korea, Japan, and China), *Microcladia californica* from the coast of California, and *Ceramium boydenii* from Korea and Japan in order to reassess the generic concept and circumscription of *Campylaephora* and to infer their phylogenetic relationships.

MATERIAL AND METHODS

Morphology

Samples were collected worldwide and sorted according to morphology under a stereomicroscope. Each identified sample was preserved in 4–5% formalin/seawater for morphological observations. Microscopic observations for developmental morphology were made on material stained with 1% aqueous aniline blue acidified with 0.1% HCl.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) with silica-gel dried specimens. The *rbc*L gene was amplified with the primer combinations F7-R753 and F645-RrbcSstart as listed in Lin *et al.* (2001) and sequenced with the primers F7, F645, F993, R376, R753, R1150, RrbcSstart (Freshwater & Rueness 1994; Lin *et al.* 2001; Gavio & Fredericq 2002). PCR and sequencing protocols were as described in Cho *et al.* (2003b). Sequences were determined for both forward and reverse strands using the ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) with the ABI Prism BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems).

Alignment and phylogenetic analyses

Generated *rbc*L sequence data were compiled (Table 1), and the sequences were manually aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI), then exported for maximum parsimony (MP) and maximum likelihood (ML) algorithms available in PAUP* (v. 4.0b10; Swofford 2003). Representatives of several genera of the Ceramieae were included in the data set. *Antithamnion* and *Scagelia* species were selected as the outgroup in the analyses.

MP trees were constructed with the heuristic search option of PAUP. Support for nodes of the MP tree was determined by calculating bootstrap proportion values (Felsenstein 1985) using 1000 replicates and randomizing the input order 500 times. For the ML analyses, the aligned sequences were first analyzed using Modeltest (v. 3.0; Posada & Crandall 1998). Support for nodes of ML tree was determined by calculating bootstrap proportion values using 100 replicates.

RESULTS

Morphological observation

***Campylaephora californica* (Farlow) T.O. Cho comb. nov.**

Figs 1–29

BASIONYM: *Microcladia californica* Farlow 1875, p. 372.

REPRESENTATIVE SPECIMENS EXAMINED: Asilomar St. Beach, Pacific Grove, Monterey Co., CA, USA (*M.H. Hommersand (M.H.H.)*, 26.vii.1988, vegetative on *Egregia*); Davenport Landing, Santa Cruz Co., CA, USA (*M.H.H.*, 9.viii.1979, male, female, and tetrasporic on *Egregia*); Davenport Landing, Santa Cruz Co., CA, USA (*M.H.H.*, 31.xii.1979, male, female, and tetrasporic on *Egregia*); Moss Beach, Monterey Co., CA, USA (*M.H. Hommersand (M.H.H.)*, 19.viii.1977, vegetative, female, and tetrasporic on *Egregia*); North Side, Horseshoe Cove, Bodega Head, Sonoma Co., CA, USA (*M.H.H.*, 4.vii.1966, female); Pigeon Point, San Mateo Co., CA, USA (*M.H.H.*, 21.xii.1992, vegetative and tetrasporic on *Egregia*).

VEGETATIVE MORPHOLOGY AND DEVELOPMENT: Thalli are dark red in color, up to 20 cm high (Fig. 1), commonly epiphytic on *Egregia menziesii*. The axes are forcipate and slightly incurved with complanate tips (Figs 2, 3). Axial cells are spherical to cylindrical, reaching 333 ± 28 × 203 ± 11 μm at the midregion of thalli. Six to seven periaxial cells are cut off obliquely from the upper end of each parent axial cell and remain at the nodes after axial cell elongation (Figs 9, 10). All periaxial cells produce corticating filaments that consolidate to form the cortex.

The cortication is complete (Fig. 4) throughout the thallus and is composed of inner and outer regions (Figs 5–8). The inner cortex is thick and consists of multiple layers of rhizoidal filaments, while the outer cortex is thin and composed of globular cells (Figs 7, 8). Five cortical initials are produced per periaxial cell in an alternate sequence, each of which bears a corticating filament (Figs 11–13). The first two cortical initials are cut off obliquely from the anterior end of each periaxial cells and grow acropetally (Figs 11, 13); the second two are produced obliquely from the posterior end and grow basipetally (Figs 12, 13). The fifth is produced on the outer face of the periaxial cell (Fig. 13). After forming the corticating filaments, the periaxial cell and inner cortical cells produce rhizoidal corticating filaments internally (Fig. 5). Internal rhizoidal filaments are more abundant in the lower than in the upper thallus parts, so that the inner cortex becomes compact and thick (Figs 6–8). Internal rhizoidal filaments between the nodes are interconnected by secondary pit connections (Fig. 5).

The branching pattern is pseudodichotomous in the same plane and irregularly alternate in mature plants (Fig. 1). Branching takes place at intervals of 4–5 (average 4.1 ± 0.4) axial cells in main axes and at intervals of 5–7 (average 5.8 ± 0.8) cells in the lateral axes. In addition, adventitious

Table 1. Taxa and collection information of samples used in the analyses of *rbc*L with their GenBank accession numbers.

Species	Collection information (location; date; *collector*)	GenBank accession no.
Antithamnion hanovioides (Sonder) De Toni	Pennington Bay, Kangaroo Island, S. Australia; 07.ix.1995; *M.H. Hommersand*	AY591927[1]
Campylaephora borealis Seo, T.O. Cho & Boo	Sinnam, Kangwon, Korea; 25.ii.1998; *T.O. Cho & H.S. Yoon* (TC002)	AY945767[2]
C. borealis	Sinnam, Kangwon, Korea; 6.vii.1999; *T.O. Cho* (TC123)	EF613499
C. californica T.O. Cho *comb. nov.*	Pigeon Point, San Mateo Co., California, USA; 21.xii.1992; *M.H. Hommersand* (TC395)	EF613500
C. crassa (Okamura) Nakamura	Gachun, Namhae, Kyengnam, Korea; 30.v.1999; *T.O. Cho & H.S. Yoon* (TC064)	EF613501
C. crassa	Gachun, Namhae, Kyengnam, Korea; 7.vii.1999; *T.O. Cho* (TC128)	EF613502
C. crassa	Gageodo, Shinan, Chunnam, Korea; 14.vii.1999; *T.O. Cho* (TC153)	EF613503
C. crassa	Gamchu, Donghae, Kangwon, Korea; 7.iv.1999; *T.O. Cho* (TC077)	EF613504
C. crassa	Sinnam, Kangwon, Korea; 25.ii.1998; *T.O. Cho & H.S. Yoon* (TC003)	AY945769[2]
C. hypnaeoides J. Agardh	Akkeshi, Hokkaido, Japan; 7.v.1999; *S.M. Boo & H.S. Yoon* (TC104)	EF613505
C. hypnaeoides	Daesado, Wando, Korea; 13.vi.1999; *T.O. Cho, & W.J. Lee* (TC146)	EF613506
C. hypnaeoides	Lopatina Cape, SW coast of Tatarskiy Strait, Sakhalin, Russia; 7.xi.2001; *R. Vadim Shtrik* (TC2942)	EF613507
C. hypnaeoides	Sonohora, Tsuyazaki, Fukuoka, Japan; 18.iii.1999; *T.O. Cho & S. Kawaguchi* (TC053)	AY945768[2]
Carpoblepharis minima E.S. Barton	Grossbuchte, Luderitz, Namibia; 9.vii.1993; *M. Hommersand* (TC408)	EF613508
Centroceras clavulatum (C. Agardh) Montagne	Punta La Cruz, Ancon, Lima, Peru; 30.viii.2003; *N. Arakaki*	DQ374331[3]
Corallophila eatoniana (Farlow) T.O. Cho, Choi, G.I.Hansen & Boo	Friday Harbor, Washington, USA; 16.vii.1998; *T.O. Cho*	AY945765[2]
Ceramium affine Setchell & N.L. Gardner	Pichilingue, Baja California, Mexico; 27.v.2000; *T.O. Cho & R.R. Rodriguez*	AF521797[4]
C. boydenii E. Gepp	Anin, Kangwon, Korea; 25.ii.1998; *T.O. Cho & H.S. Yoon*	AY945781[2]
C. boydenii	Heuksando, Shinan, Chunnam; 18.vii.1999; *T.O. Cho* (TC182)	EF613509
C. boydenii	Jindo, Chunnam, Korea, 4.v.1997; *H.S. Yoon* (TC027)	EF613510
C. boydenii	Tsuyazaki, Fukuoka, Japan; 16.iii.1999; *T.O. Cho & S. Kawaguchi* (TC057)	EF613511
C. codicola J. Agardh	Boiler Bay, Oregon, USA; 8.vii.1998; *T.O. Cho & G.I. Hansen*	AY155522[5]
C. horridum Setchell & N.L. Gardner	San Juan De La Costa, Baja California, Mexico; 15.vi.2000; *T.O. Cho & R.R. Rodriguez*	AF521796[4]
C. inkyuii T.O. Cho, Fredericq & Boo	Guryongpo, Pohang, Korea; 18.v.1999; *T.O. Cho & H.S. Yoon*	AF521800[4]
C. interruptum Setchell & N.L. Gardner	Dana Point, California, USA; 4.xii.1999; *T.O. Cho & S. Murray*	AY155527[5]
C. japonicum Okamura	Namhae, Kyengnam, Korea; 3.iii.1998; *T.O. Cho*	AY945783[2]
C. kondoi Yendo	Stoneman, Qingdao, China; 26.vi.1994; *M.H. Hommersand* (TC430)	EF613512
C. kondoi	Songbang, Tongyoung, Korea; 30.iii.1998; *T.O. Cho & Y.S. Oh*	AY945782[2]
C. paniculatum Okamura	Guryongpo, Pohang, Kyengbuk, Korea; 18.v.1998; *T.O. Cho & H.S. Yoon* (TC025)	EF613513
C. sinicola Setchell & N.L. Gardner	Dana Point, California, USA; 4.xii.1999; *T.O. Cho & S. Murray*	AY155531[5]
Herpochondria elegans (Okamura) Itono	Jeongdori, Wando, Korea; 15.xii.1998; *S.M. Boo, T.O. Cho & H.G. Choi*	AY945771[2]
Microcladia borealis Ruprecht	Otter Crest, Oregon, USA; 12.vii.1998; *T.O. Cho & G.I. Hansen* (TC004)	AY945770[2]
M. borealis	Lone Ranch Beach, Brookings, Josephine Co., Oregon, USA; 18.v.1999; *M.H. Hommersand* (TC391)	EF613514
Reinboldiella schmitziana (Reinbold) De Toni	Guryongpo, Pohang, Korea; 4.v.1998; *T.O. Cho & H.S. Yoon*	AY945772[2]
Scagelia occidentalis (Kylin) E.M. Wollaston	Washington, USA; s.d., *P.W. Gabrielson* #877	AY945763[2]

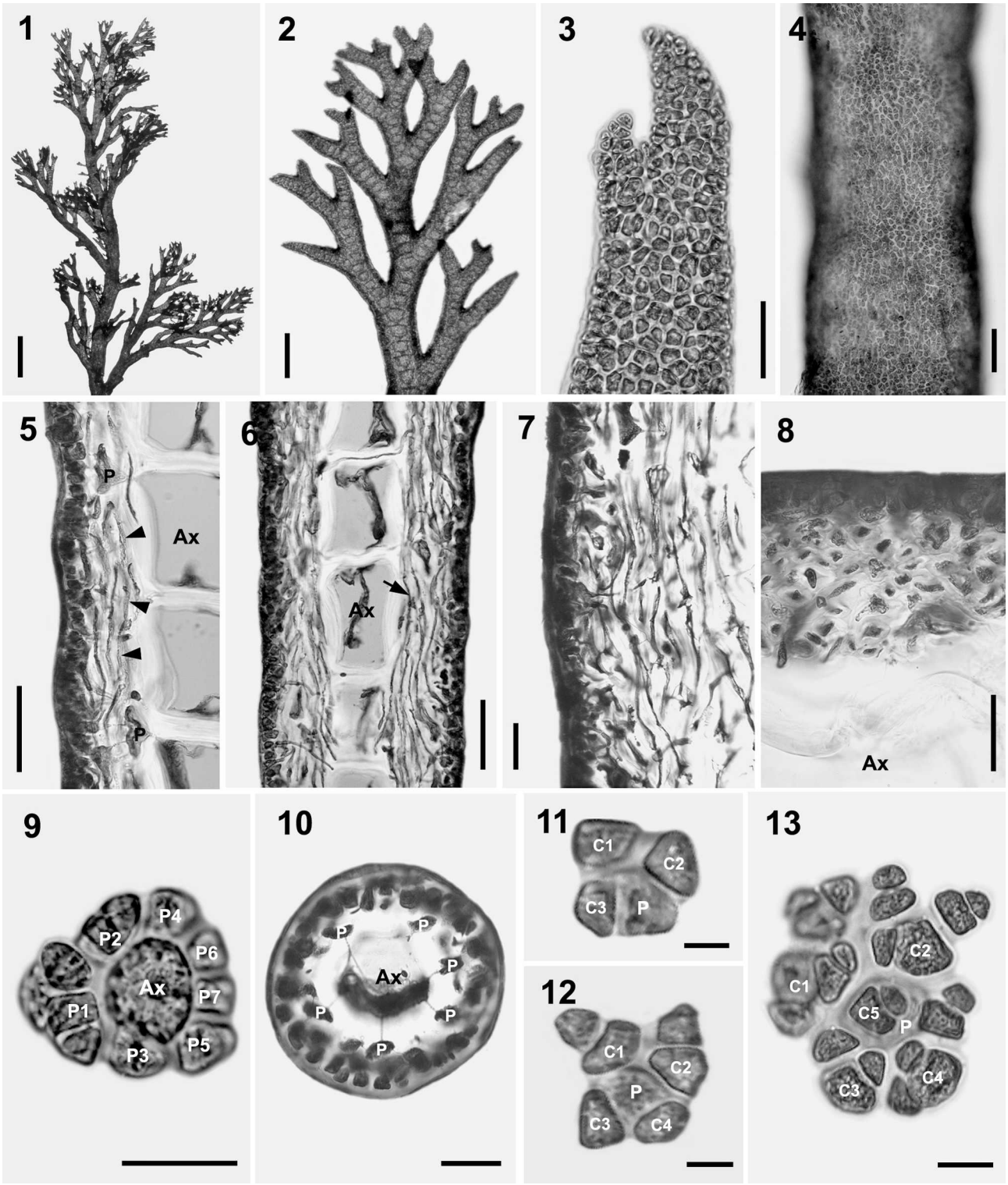
[1] From Cho *et al.* (2005).
[2] From Cho *et al.* (2008).
[3] From Won *et al.* (2007).
[4] From Cho *et al.* (2003a).
[5] From Cho *et al.* (2003b).

branches are produced from periaxial cells. They are small, curved, and abundant along the adaxial side or may occur on all sides of branches. The holdfast is a conical disc composed of rhizoidal cells at the base that attaches to the surface of the host.

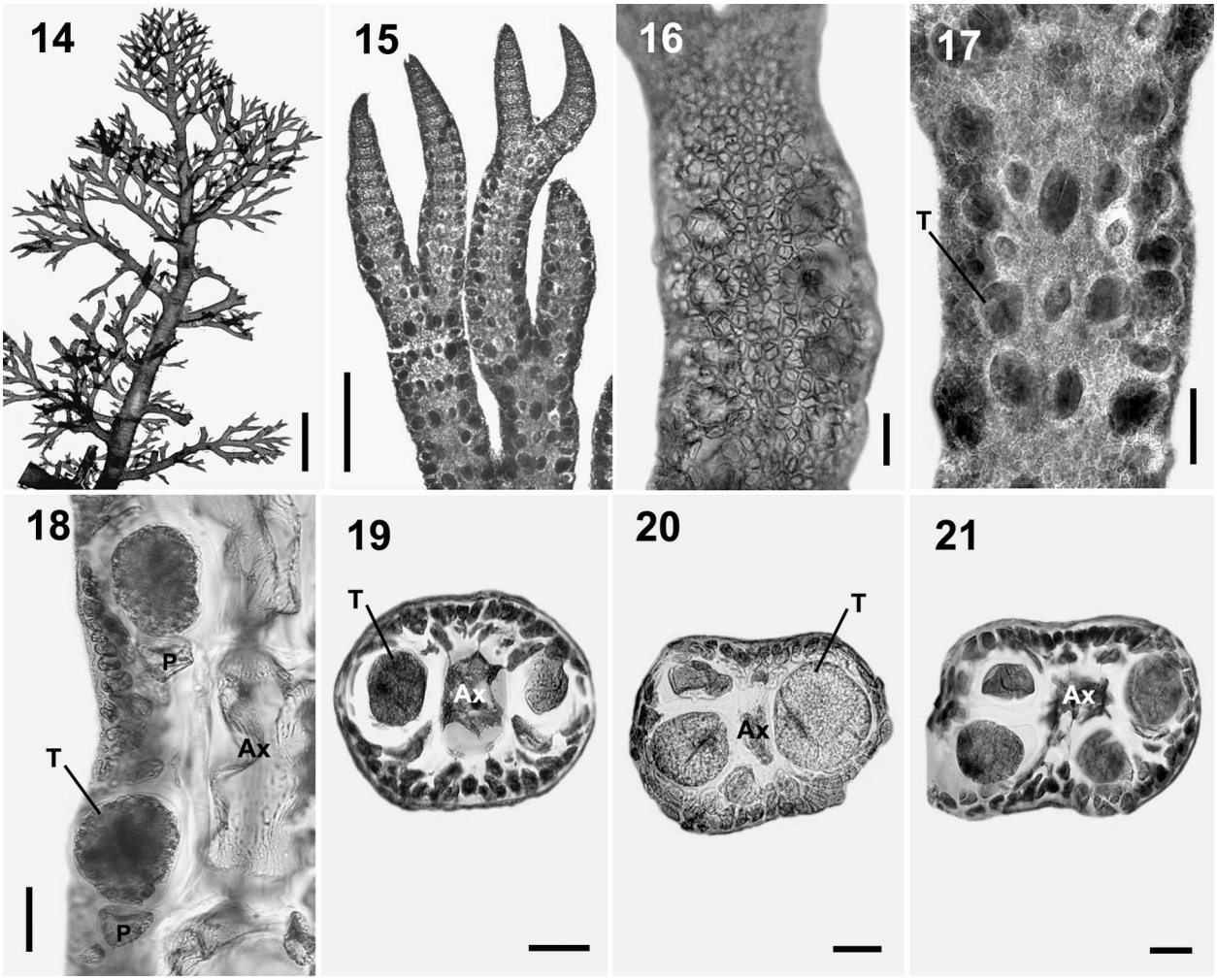
REPRODUCTIVE MORPHOLOGY AND DEVELOPMENT: **Tetrasporangial plants.** In tetrasporangial thalli (Fig. 14), tetrasporangia are completely immersed in the cortex (Fig. 16), whorled at the nodes in the upper branches (Fig. 15), and scattered irregularly in the midregion (Fig. 17). They are initially produced from opposite periaxial cells in the upper parts (Figs 16, 18–21) and later from inner cortical cells in the midregion (Fig. 17). Tetrasporangia are cruciately divided, spherical to ellipsoidal, and 96.4 ± 3.8 μm × 80.8 ± 2.7 μm with sheaths and 81.6 ± 6.6 μm × 74.1 ± 4.4 μm without sheaths.

Male plants. Spermatangia are first produced adaxially and later in a whorl around the axis, forming a cushion that covers the entire cortical surface (Figs 22, 23). Spermatangial parent cells develop from outer cortical cells and produce one to two spermatangia terminally (Fig. 24). Spermatangia are colorless and elliptical to spherical, measuring 4 ± 1 × 3 ± 1 μm in size.

Female plants. In female thalli, procarps are produced in a row on the abaxial side of both branches and branchlets



Figs 1–13. Vegetative structures (LAF-21-07-1992: slides 1, 2, 4, and 5) of *Campylaephora californica* (Farlow) T.O. Cho comb. nov.
Fig. 1. Vegetative thallus. Scale bar, 2 mm.
Fig. 2. Upper part showing alternate branching pattern. Scale bar, 0.25 mm.
Fig. 3. Apical region. Scale bar, 40 μm.
Fig. 4. Complete cortication in middle part of thallus. Scale bar, 100 μm.
Fig. 5. Longitudinal section of upper thallus part showing internal rhizoidal filaments (arrow heads) linked by secondary pit connections. Scale bar, 100 μm.
Figs 6–7. Longitudinal sections showing the development of the multilayered internal rhizoidal filaments (arrow) in middle (6) and lower (7) parts of thallus. Scale bars, 100 μm (6), 50 μm (7).
Fig. 8. Cross section through an internode showing internal rhizoidal filaments in lower parts of thallus. Scale bar, 40 μm.



Figs 14–21. Tetrasporangial structures (LAF-09-08-1979: slides 1, 2, and 5) of *Campylaephora californica* (Farlow) T.O. Cho comb. nov.
Fig. 14. Tetrasporangial thallus. Scale bar, 2 mm.
Fig. 15. Apex of tetrasporangial thallus. Scale bar, 0.25 mm.
Fig. 16. Terminal region showing arrangement of the tetrasporangia. Scale bar, 100 μm.
Fig. 17. Mid-region showing tetrasporangia scattered irregularly in whorls. Scale bar, 100 μm.
Fig. 18. Longitudinal section of upper thallus showing tetrasporangia produced from periaxial cells. Scale bar, 50 μm.
Figs 19–21. Cross sections showing opposite arrangement of successive tetrasporangia. Scale bars, 50 μm.
Abbreviations: T, tetrasporangium. Other abbreviations as in Figs 1–13.

(Figs 25, 28, 29). Young carposporophytes are situated inside the cortex and become swollen as they mature (Fig. 27). Mature carposporophytes are protected by the outer cortical cells, and involucral branchlets are absent (Fig. 28). They are spherical and 189 ± 43 μm long and 232 ± 51 μm in diameter (Figs 28, 29).

Several morphological states in the type species of *Campylaephora*, *C. hypnaeoides*, are shown in Figs 30–42 for comparison with *C. californica*.

Ceramium boydenii E. S. Gepp

Figs 43–67

REPRESENTATIVE SPECIMENS EXAMINED: Anin, Kangwon, Korea (*T.O. Cho (T.O.C) & H.S. Yoon (H.S.Y.)*, 25.ii.1998, vegetative); Bogildo, Chunnam, Korea (*T.O.C.*, 26.vii.2000, vegetative); Gachun Kyungnam, Korea (*T.O.C.*, 7.vii.1999, male, female, and tetrasporic); Jindo, Chunnam, Korea (*H.S.Y.*, 4.v.1997, vegetative); Heuk-

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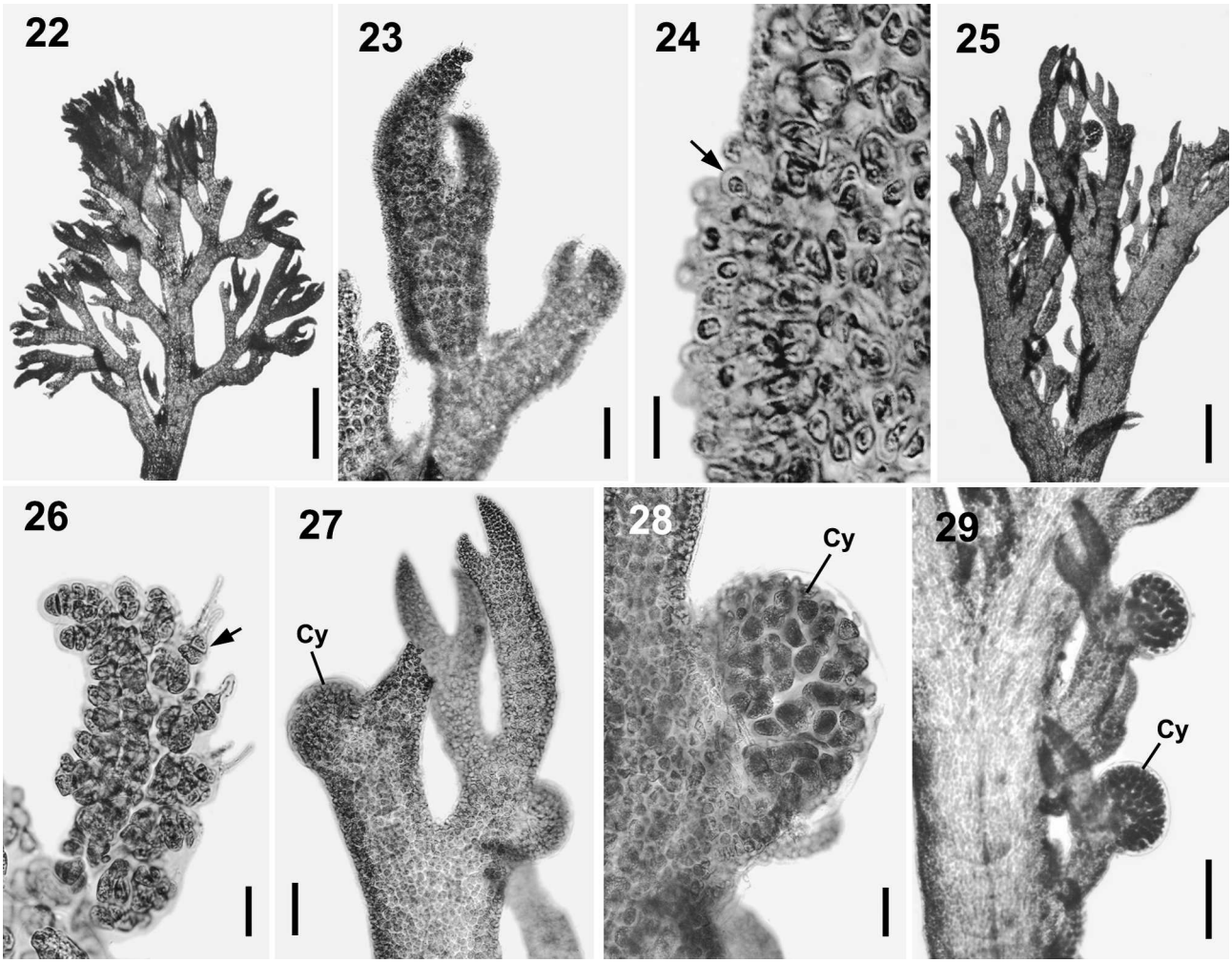
Fig. 9. Cross section of young thallus showing alternate sequence of periaxial cell formation from axial cell. Scale bars, 40 μm.
Fig. 10. Cross section through the node of a mature thallus. Scale bars, 40 μm.
Figs 11–13. Corticating filaments showing development of five cortical initials. Scale bars, 5 μm (11, 12), 10 μm (13).
Abbreviations: Ax, axial cell; C1-5, cortical initials numbered by sequence of formation; P, periaxial cell (P1, P2, P3, and so on indicate the production sequence of the periaxial cell).



Figs 22–29. Male (LAF-09-08-1979: slide 6) and female (LAF-09-08-1979: slides 8, 9, and 10) structures of *Campylaephora californica* (Farlow) T.O. Cho comb. nov.
Fig. 22. Male thallus. Scale bar, 0.5 mm.
Fig. 23. Apex of male thallus. Scale bar, 50 μm.
Fig. 24. Spermatangia (arrow) derived from spermatangial parent cells. Scale bar, 10 μm.
Fig. 25. Female thallus. Scale bar, 0.5 mm.
Fig. 26. Procarps (arrow) on abaxial side of branch near apex. Scale bar, 20 μm.
Fig. 27. Young carposporophytes protected by outer cortical cells without involucral branchlets. Scale bar, 100 μm.
Fig. 28. Mature carposporophyte near apex. Scale bar, 50 μm.
Fig. 29. Mature carposporophytes on branchlets. Scale bar, 0.25 mm.
Abbreviations: Cy, cystocarp. Other abbreviations as in Figs 1–13.

sando, Shinan, Chunnam, Korea (*T.O.C.*, 18.vii.1999, vegetative); Tsuyazaki, Fukuoka, Japan (*T.O.C. & S. Kawaguchi*, 16.iii.1999, vegetative).

VEGETATIVE MORPHOLOGY AND DEVELOPMENT: Thalli are tough, rose red in color, up to 15–24 cm high (Fig. 43), and epiphytic on *Sargassum* spp. or other seaweeds. The axes bear slightly forcipate, strongly curved tips (Fig. 44). Axial cells are spherical to cylindrical, reaching 199 ± 12 × 145 ± 6 μm at the level of the seventh dichotomy below the apex. Seven to eight periaxial cells are cut off obliquely from the upper end of each parent axial cell and remain at the nodes after axial cell elongation (Figs 52, 53). All periaxial cells produce corticating filaments that form the cortex.

Cortication is complete throughout the thallus (Fig. 46), composed of inner and outer regions (Figs 47–51). The

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Figs 30–42. Vegetative (LAF-18-03-1999: slides 1, 3, and 4) and tetrasporangial (LAF-18-03-1999: slides 6 and 7) structures of *Campylaephora hypnaeoides* J. Agardh.
Fig. 30. Apex showing sickle-shaped terminal portion, a key character of the species. Scale bar, 1 mm.
Fig. 31. Complete cortication in middle part of thallus, Scale bar, 100 μm.
Fig. 32. Longitudinal section of upper thallus. Scale bar, 50 μm.

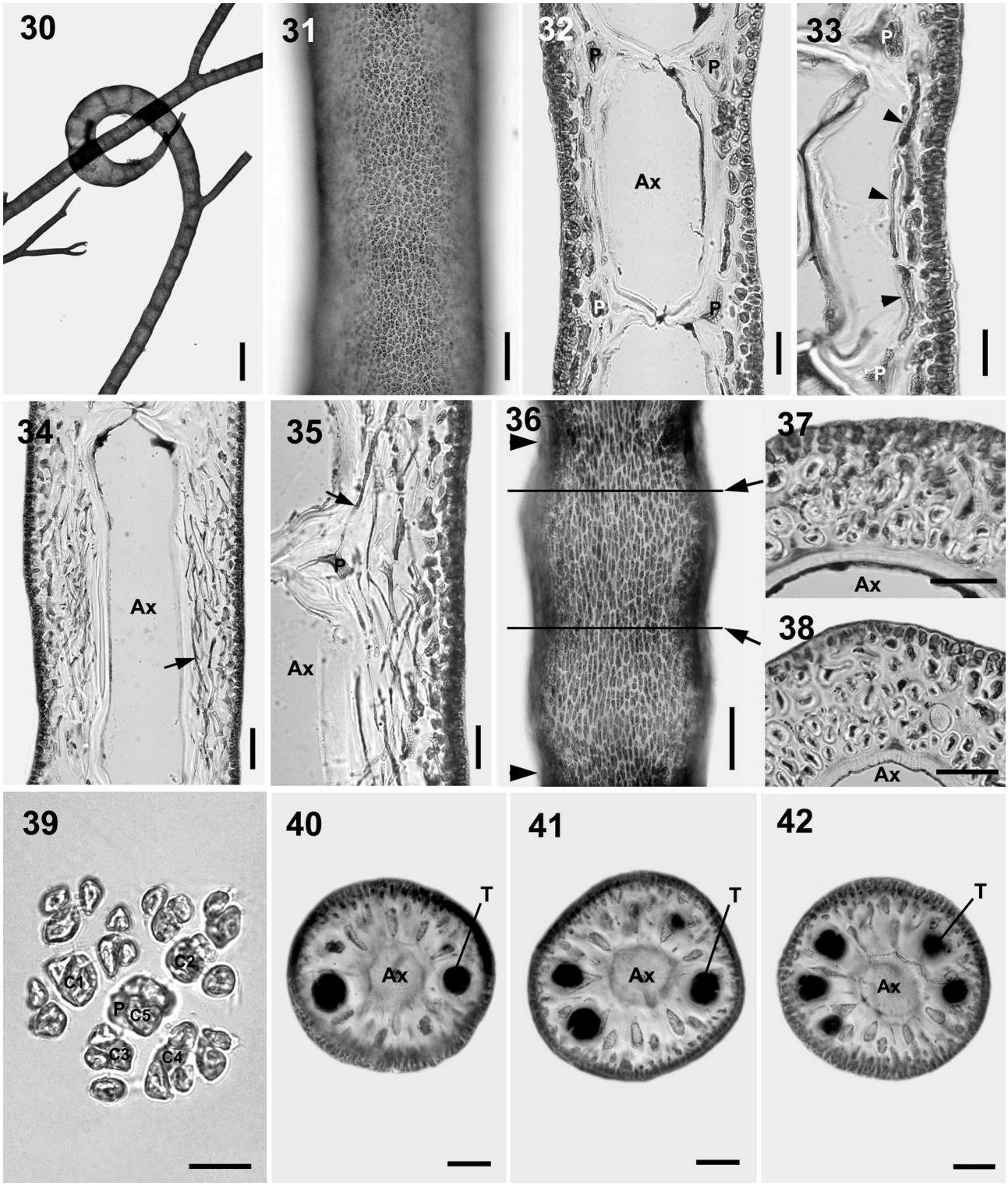
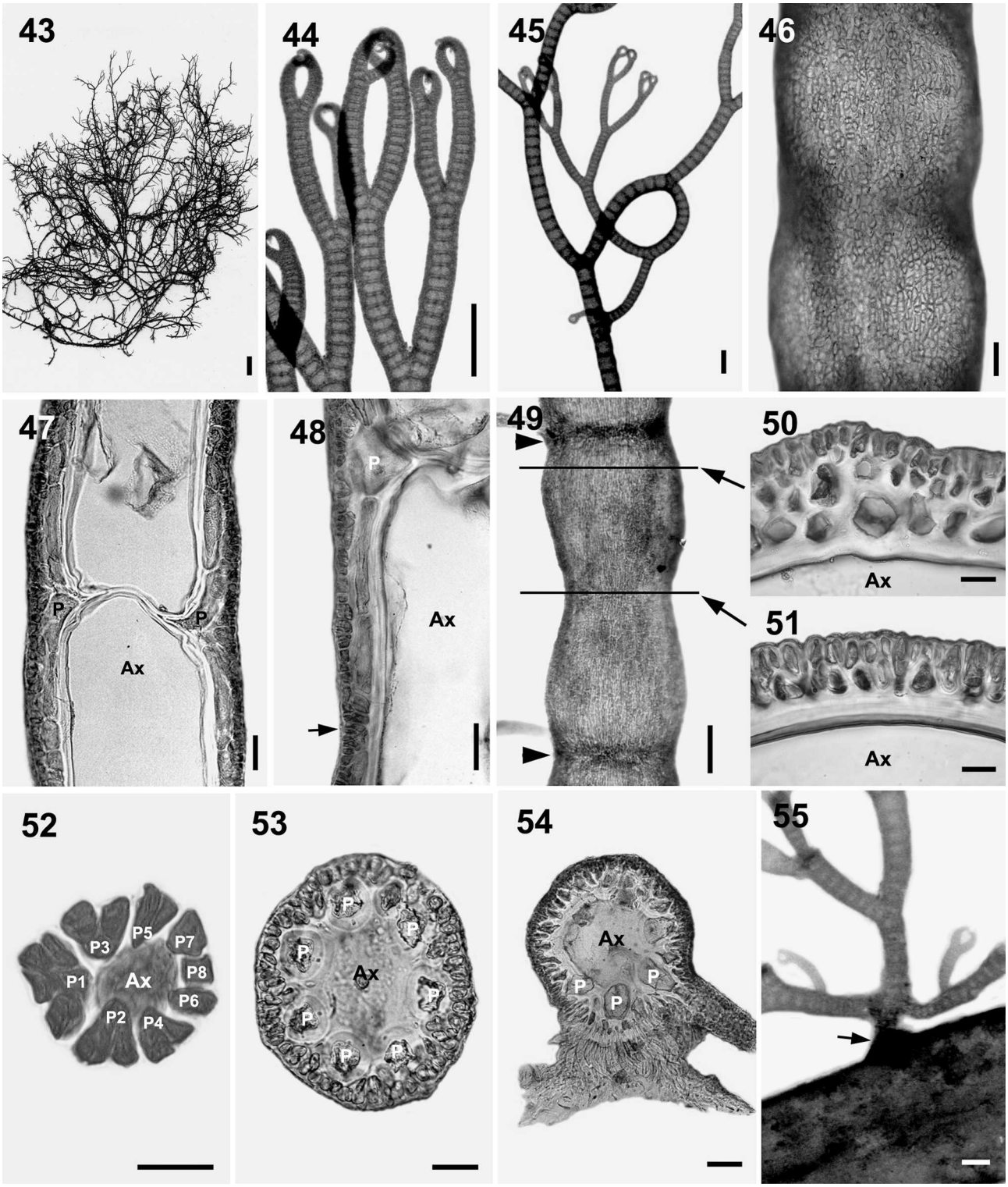


Fig. 33. Longitudinal section of upper thallus part showing internal rhizoidal filaments (arrow heads) linked by secondary pit connections. Scale bar, 50 μm.
Figs 34, 35. Longitudinal section of middle (34) and lower (35) parts of thallus part showing multilayered internal rhizoidal filaments (arrows). Scale bars, 50 μm.
Fig. 36. A view between the nodes (arrow heads). Scale bar 0.2 mm.
Figs 37, 38. Cross sections near node (37) and the center of internode (38) on lower thallus part. Scale bars, 50 μm (37), 20 μm (38).
Fig. 39. Cortication consisting of five corticating filaments. Scale bars, 20 μm.
Figs 40–42. Cross sections through the node showing opposite arrangement of successive tetrasporangia. Scale bars, 100 μm.
Abbreviations: As in Figs 1–13 and 14–21.



Figs 43–55. Vegetative structures (LAF-25-02-1999: slides 1, 3, 4, and 5) of *Ceramium boydenii* E. S. Gepp.
Fig. 43. Vegetative thallus. Scale bar, 3 mm.
Fig. 44. Apical regions. Scale bar, 0.5 mm.
Fig. 45. Upper thallus with branch coiling around host. Scale bar, 0.5 mm.
Fig. 46. Complete cortication in middle part of thallus. Scale bar, 50 μm.
Fig. 47. Longitudinal section showing the single-layered and elongated inner cortical cells in middle parts of thallus. Scale bars, 50 μm.
Fig. 48. Longitudinal section showing the break (arrow) between the descending and ascending filaments. Scale bars, 50 μm.
Fig. 49. A view between the nodes (arrowheads). Scale bar, 0.25 mm.
Figs 50, 51. Cross sections at node (50) and internode (51) of lower thallus part. Scale bars, 20 μm.
Fig. 52. Cross section of young thallus showing alternate sequence of periaxial cell formation from axial cell. Scale bar, 20 μm.

inner cortex is thin and consists of a single layer of elongated cells, whereas the outer cortex is composed of globular cells (Figs 47, 48). Five cortical initials are produced per periaxial cell in an alternate sequence and bear the corticating filaments. The first two cortical initials are cut off obliquely from the anterior ends of the periaxial cells and grow acropetally; the second two are produced obliquely from the posterior ends and grow basipetally. The fifth initial is cut off from the outer face of the periaxial cell. Inner cortical cells produced from the periaxial cells elongate and form a single layer throughout the mature thallus (Figs 47, 48). They are longer and wider at the nodes than in the region between nodes (Figs 48, 50, 51). The outer cortical cells are small and angular or globular.

The branching pattern is pseudodichotomous in the same plane (Figs 43, 44). Branching takes place at intervals of 8–15 axial cells in the main axes and at intervals of 9–16 cells in the lateral axes. Adventitious branches are produced from periaxial cells along opposite sides or on all sides of branches. They are small, curved, and abundant in the lower parts of the thallus. Gland cells are angular and $4 \pm 1 \times 8 \pm 1$ μm in size. They develop from outer cortical cells and are dispersed in the cortex. The holdfast is a conical disc composed of rhizoidal cells near the base that attaches to the surface of the host (Fig. 55). Some branches wind around a host and are attached to it by rhizoids produced from superficial cells (Fig. 54).

REPRODUCTIVE MORPHOLOGY AND DEVELOPMENT: **Tetrasporangial plants.** In tetrasporangial thalli (Fig. 56), tetrasporangia are completely immersed in the cortex and arranged in two rows along both sides of the axes in the upper branches and adventitious branchlets (Figs 57–59). They are first formed on the abaxial sides and then on the adaxial sides of branches (Figs 57, 58, 60, 61). Tetrasporangia are cruciately divided, spherical to ellipsoidal, and 95 ± 7 μm $\times 90 \pm 9$ μm with sheaths and 110 ± 14 μm $\times 100 \pm 9$ μm without sheaths.

Male plants. Male thalli are small, about 2 cm high and regularly dichotomous and bear numerous adventitious branchlets (Fig. 62). Spermatangia are first produced adaxially and later form in whorls around the apices of the axes and adventitious branchlets, producing a cushion that covers the entire cortical surface (Figs 63, 64). Spermatangial parent cells develop from outer cortical cells and produce one to two spermatangia terminally (Fig. 65). Spermatangia are colorless and elliptical to spherical, measuring $4 \pm 1 \times 3 \pm 1$ μm in size.

Female plants. Female thalli with carposporophytes (Fig. 66) are small, about 1–2 cm high and regularly dichotomous, and bear numerous adventitious branchlets on the adaxial side. Carposporophytes are produced on both branches and branchlets. Mature carposporophytes with five to six involucral branchlets are spherical, 400 ± 15 μm long, and 370 ± 25 μm in diameter (Fig. 67).

Phylogenetic analysis based on *rbc*L

A 1418-bp portion of the 1467-bp *rbc*L gene (96.7% nucleotides sequenced) was selected that included 299 parsimony informative sites, excluding the outgroup. Interspecies pairwise comparison of the *rbc*L sequences revealed that *Campylaephora californica*, previously known as *Microcladia californica*, showed lower gene sequence divergences (3.0–3.9%) compared to *Campylaephora* species than the divergence seen with *Microcladia borealis* Ruprecht (9.4%). *Ceramium boydenii* exhibited 4.2–5.8% gene sequence divergences compared to *Campylaephora* species.

An MP phylogenetic tree (Fig. 68) was obtained from the alignment of the *rbc*L sequences. The topologies of MP and ML *rbc*L trees were congruent except for the phylogenetic position of *Carpoblepharis* and *Reinboldiella*. The genus *Campylaephora* was monophyletic in both MP and ML analyses with robust bootstrap support (97% in MP, 99% in ML). *Campylaephora californica* nested within the *Campylaephora* clade in all *rbc*L phylogenetic trees, was separate from *Microcladia borealis*, and situated alongside *Campylaephora borealis* in a weak clade that was poorly resolved ($< 50\%$). All samples of *Campylaephora crassa* from different localities grouped in a well-supported clade, while those of *Campylaephora hypnaeoides* formed slightly different lineages. *Ceramium boydenii* was basal to the clade containing all *Campylaephora* species (Fig. 68).

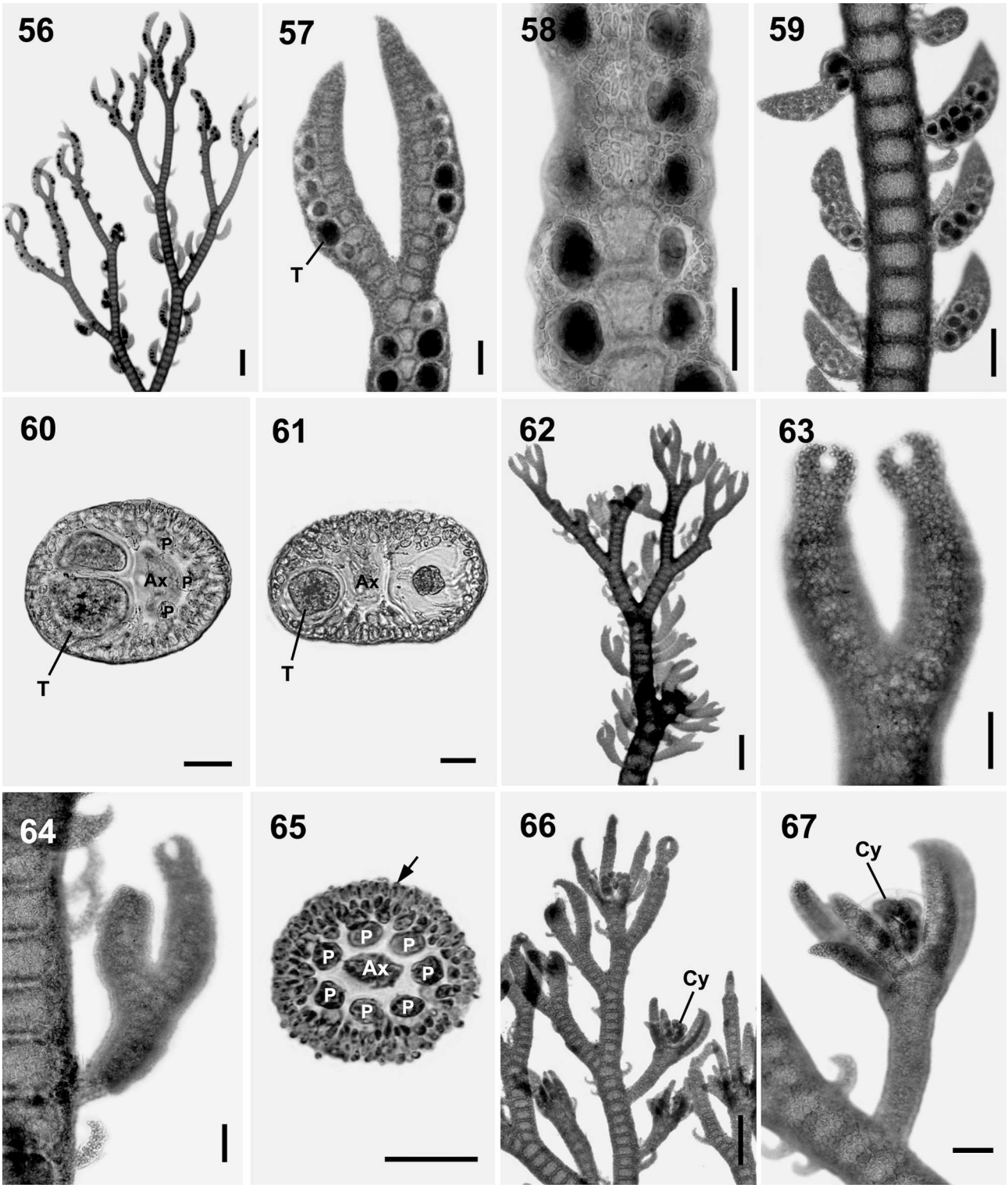
DISCUSSION

Our morphological and molecular observations suggested that the genus *Campylaephora* should be redefined to include species having internal rhizoidal filaments derived from periaxial and cortical cells that are connected laterally by secondary pit connections. *Microcladia californica* is recognized as *Camyplaephora californica* (Farlow) T.O. Cho comb. nov. The species of *Campylaephora* form a monophyletic clade in all molecular phylogenetic analyses based on *rbc*L sequences.

Although the genus *Campylaephora* has been characterized by the presence of internal rhizoidal filaments, a thick cortex, hooked terminal branches, discoid holdfasts, dichotomous ramification, sickle-shaped terminal and lateral branchlets, and unusually large tetrasporangia (J. Agardh 1851; Schmitz & Hauptfleisch 1897; Nakamura 1950), of these, internal rhizoidal filaments and a thick cortex are the critical diagnostic characters of the genus. Nakamura (1950, p. 166) mentioned that the rhizoidal cells in the cortex are secondarily formed from cortical cells and

←

Fig. 53. Cross section through node of mature thallus. Scale bar, 50 μm.
Fig. 54. Cross section of cortical node with rhizoids on a branch winding around the host. Scale bar, 100 μm.
Fig. 55. Holdfast (arrow) attached to the host. Scale bar, 0.5 mm.
Abbreviations: As in Figs 1–13.



Figs 56–67. Tetrasporangial (LAF-07-07-1999: slides 2 and 3), male (LAF-07-07-1999: slides 4 and 6), and female structures (LAF-07-07-1999: slide 8) of *Ceramium boydenii* E. S. Gepp.

Fig. 56. Tetrasporanigal thallus. Scale bar, 0.5 mm.
Fig. 57. Apex of tetrasporangial thallus bearing abaxial tetrasporangia. Scale bar, 100 μm.
Fig. 58. Upper part of tetrasporangial thallus showing oppositely arranged tetrasporangia. Scale bar, 100 μm.
Fig. 59. Middle part of tetrasporangial thallus with adventitious branchlets on both sides. Scale bar, 200 μm.
Figs 60, 61. Development of tetrasporangia showing the opposite sequence of their formation. Scale bars, 50 μm.
Fig. 62. Male thallus. Scale bar, 0.5 mm.
Fig. 63. Apex of male thallus. Scale bar, 100 μm.
Fig. 64. Adventitious branchlet with spermatangia. Scale bar, 100 μm.

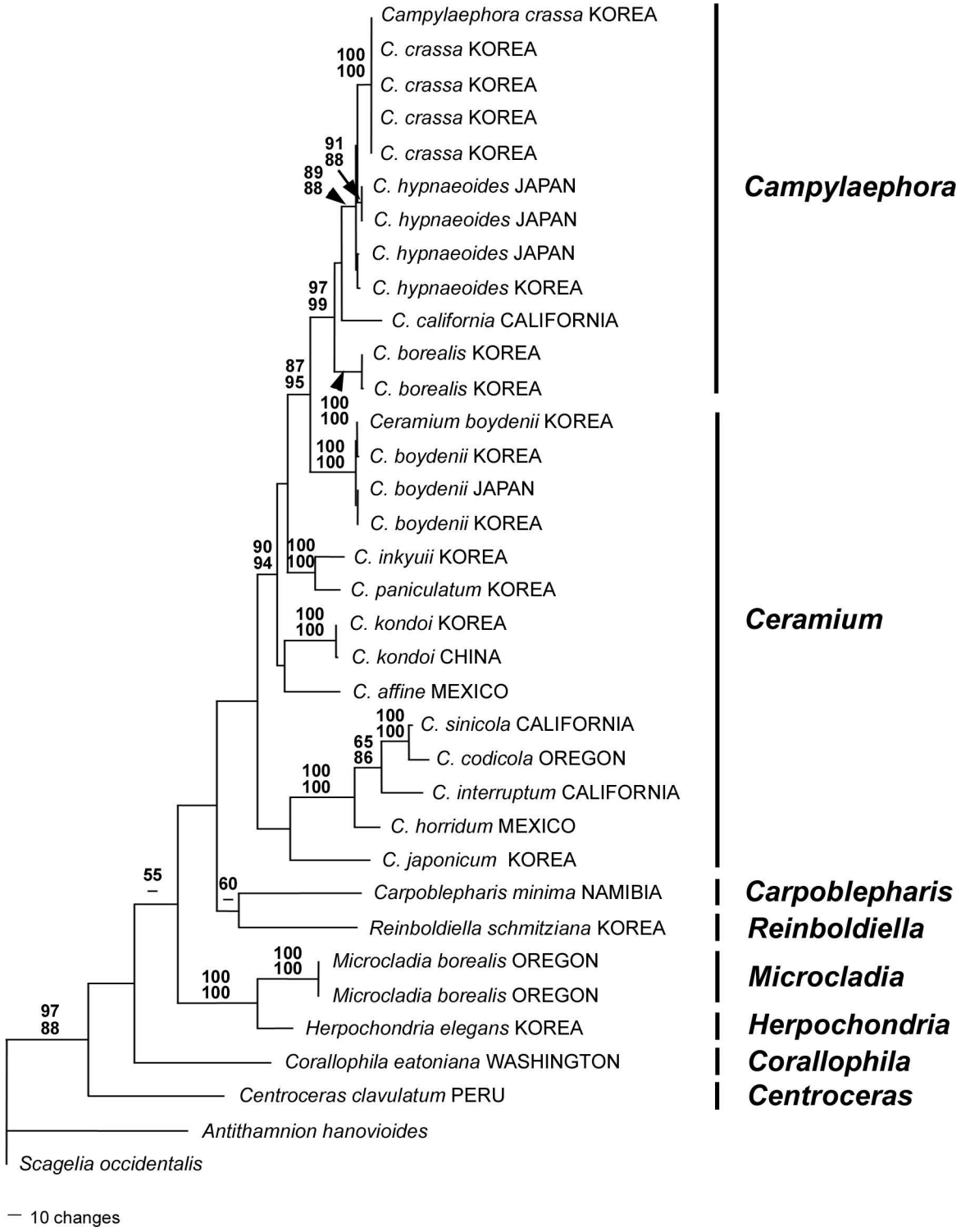


Fig. 68. One of 18 most parsimonious trees from MP analysis of the chloroplast *rbc*L sequence data (length = 1175 steps, consistency index = 0.465, retention index = 0.649, and rescaled consistency index = 0.301). Bootstrap proportion values (> 50%) for MP (top) and ML (bottom) are shown at the nodes.

←

Fig. 65. Cross section through the node with spermatangia (arrow). Scale bar, 50 μm.
Fig. 66. Female thallus. Scale bar, 0.3 mm.
Fig. 67. Mature carposporophyte with involucral branchlets. Scale bar, 100 μm.
Abbreviations: As in Figs 1–13, 14–21, and 22–29.

are not merely elongated cortical cells but true rhizoidal cells. Internal rhizoidal filaments have been observed in all *Campylaephora* species including the type species, *C. hypnaeoides* (Nakamura 1950; Seo *et al.* 2003). Our results show that they are initially slender and filamentous, develop as true rhizoidal filaments from the start, and form a multilayered thick inner cortex that is distributed throughout the thallus except near apex (Figs 5–7, 32–35). Rhizoidal filaments have also been reported in some species of *Ceramium*, such as *C. boydenii* (Gepp 1904, p. 164), *C. personatum* Setchell & N.L. Gardner (1930, p. 171), *C. nitens* (C. Agardh) J. Agardh (1851, p. 130), and *C. californicum* J. Agardh (1894, p. 45). Of these species, *Ceramium boydenii* and *C. nitens* are similar to *Campylaephora* in thallus appearance (Cho *et al.* 2004); however, they are distinguished from *Campylaephora* in that the cortex consists of a single layer (Fig. 47), having thin cell walls (Fig. 50) and cortical filaments that lack secondary pit connections.

Of the key characters for *Campylaephora*, the dichotomous ramification, the sickle-shaped or hooked terminal branches, the large tetraspores, and the discoid holdfast may not be available as key characters for *Campylaephora* because these are shared with some species of *Ceramium*. The dichotomous ramification is a common character in both *Campylaephora* and *Ceramium*, the sickle-shaped or hooked terminal branches are also present in *Ceramium boydenii*, and the large size of the tetrasporangia overlaps with those found in some *Ceramium* species. Nakamura (1954) distinguished the disc-forming holdfast of *Campylaephora* from that of *Ceramium*, which consists of a cluster of rhizoids, and Seo *et al.* (2003) considered the disc-forming holdfast as a putative autoapomorphy for *Campylaephora*; however, this character is shared with *Ceramium boydenii* (Nakamura 1954) and *Ceramium kondoii* Yendo (Boo & Yoon 1993). Therefore, the key character for *Campylaephora* should be restricted to the multiple layers of rhizoidal filaments that are linked by secondary pit connections.

We observed the thick and compact inner cortex composed of multilayered rhizoidal filaments interconnected by secondary pit connection in both *Campylaephora californica* and *Campylaephora hypnaeoides*, the type species. Secondary pit connections are regarded as one of the diagnostic characters of *Campylaephora* (Seo *et al.* 2003; Cho *et al.* 2004); these structures as shown here are absent in *Ceramium boydenii*. Seo *et al.* (2003) also observed secondary pit connections in *Campylaephora borealis* and considered them as being autapomorphies for the genus. In our *rbc*L sequence analyses, all *Campylaephora* species form a monophyletic clade in all the molecular analyses based on *rbc*L sequence data, and they are supported by the presence of multilayered rhizoidal filaments cross-linked by secondary pit connections. This is congruent with previous molecular trees based on the RuBisco spacer region and *psb*A by Seo *et al.* (2003). *Campylaephora californica* nests in the clade containing the other *Campylaephora* species and is separate from *Microcladia borealis*, another species found in the northeast Pacific. *Camyplaephora californica* is recognized as an independent species within *Campylaephora* based on the *rbc*L gene sequence analysis and is distinguished morphologically by the absence of involucral branchlets associated with the carposporophyte and its specific basiphyte, *Egregia menziesii*. Although *Campylaephora borealis* and *C. hypnaeoides* have been shown to have a close relationship in RuBisCo spacer and *psb*A gene analyses (Seo *et al.* 2003), our *rbc*L sequence data demonstrate that *C. californica* is most closely related to *C. borealis*. Although the taxonomic distinction between *Campylaephora californica* (as *Microcladia californica*) and *Microcladia coulteri* Harvey was unclear in the literature (Hommersand 1963; Abbott & Hollenberg 1976), Gonzalez & Goff (1989a) showed that these two species are physiologically and morphologically distinct based on their morphology, phenology, basiphyte range, and behavior in culture and in hybridization experiments.

Campylaephora californica is an endemic species in California (Abbott & Hollenberg 1976). Our *rbc*L sequence data also indicate that *C. californica* is more closely related to *C. borealis*, a species adapted to colder-water environments (Seo *et al.* 2003), than to the other *Campylaephora* species. These distributional and molecular data suggest that *C. californica* originated from an ancestor related to *C. borealis* found in the northern regions that later adapted to the temperate climate of central California. In nature, *C. californica* grows only on the brown alga *Egregia menziesii* (Gonzalez & Goff 1989a), whereas *C. borealis* grows on gigartinalean and ceramialean Rhodophyta (Seo *et al.* 2003); *C. crassa* grows on *Phyllospadix*, *Rhodomela*, and *Sagassum* (Nakamura 1950); and *C. hypnaeoides* grows on *Sagassum* and *Laminaria* (Nakamura 1950). Although the genus *Caympylaephora* is an endemic reported only in the northwest Pacific (e.g., Nakamura 1954; Noda 1974; Seo *et al.* 2003), our data show that the area of its distribution should be expanded to include the northeast Pacific.

According to Cho *et al.* (2008), *Ceramium* is polyphyletic. The splitting of *Ceramium* into a series of monophyletic genera has started with the proposal of *Gayliella* T.O. Cho, Boo, Hommersand, Maggs, McIvor & Fredericq (Cho *et al.* 2008). More taxa need to be included in the data set to correctly identify the generic position of *C. boydenii*, a taxon that is currently paraphyletic. We restricted the assignment of *Campylaephora* to taxa above the 97/99-bp node because *C. boydenii* does not possess the key morphological diagnostic characters of *Campylaephora*.

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