Coastal Marine Science 30(1): 209–213, 2006

Preliminary report on the molecular phylogeny of the *Laurencia* complex (Rhodomelaceae)

Tsuyoshi ABE[1]*, Akira KURIHARA[2], Shigeo KAWAGUCHI[3], Ryuta TERADA[4] and Michio MASUDA[2]

[1] *The Hokkaido University Museum, Hokkaido University, Sapporo 060–0810, Japan*
[2] *Graduate School of Science, Hokkaido University, Sapporo 060–0810, Japan*
[3] *Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan*
[4] *Faculty of Fisheries, Kagoshima University, Kagoshima 890–0056, Japan*
**E-mail: tabe@museum.hokudai.ac.jp*

Received: 26 August 2005; Accepted: 10 November 2005

Abstract—A molecular phylogenetic analysis of three closely related red algal genera, *Laurencia*, *Chondrophycus* and *Osmundea* (Rhodomelaceae) that are often called the *Laurencia* complex or *Laurencia* sensu lato, mainly of the north-western Pacific species, was carried out based on *rbc*L gene sequences. The sequence data downloaded from GenBank were also included in our analysis. The monophyly of the clade of *Osmundea* species was supported by high bootstrap value. All examined species of *Laurencia* sensu stricto with typical features (four periaxial cells per vegetative axial segment, longitudinally oriented secondary pit-connections between contiguous superficial cortical cells, *corps en cerise* within superficial cortical and trichoblast cells) also constituted a monophyletic clade with high bootstrap value. On the other hand, *Chondrophycus* species were divided into two separated clades with relatively high bootstrap values respectively. Furthermore, *L. flexilis*, which has four periaxial cells per vegetative axial segment but lacks secondary pit-connections between contiguous superficial cortical cells, constituted an independent monophyletic clade with high bootstrap value. These results suggest that only two groups, *Osmundea* and the typical *Laurencia*, are monophyletic and *Chondrophycus* is polyphyletic within the *Laurencia* complex. The phylogenetic position of *L. flexilis*, an intermediate species between the typical *Laurencia* and *Chondrophycus*, must be clarified to determine the key features to distinguish *Chondrophycus* and *Laurencia* sensu stricto. Further investigations based on the other genes are expected.

Key words: *Chondrophycus*, *corps en cerise*, *Laurencia*, *Laurencia* complex, *L. flexilis*, molecular phylogeny, periaxial cell, *rbc*L

Introduction

Based on vegetative and reproductive features, the red algal genus *Laurencia* Lamouroux (1813) was recently separated into three genera: *Laurencia*, *Chondrophycus* (Tokida et Saito) Garbary et Harper (1998) and *Osmundea* Stackhouse (1809) (Garbary & Harper 1998, Nam 1999). These closely related genera, which are often called the *Laurencia* complex or *Laurencia* sensu lato, are unique in the Rhodomelaceae by the following features: apical cells are sunk in apical pits of axes and branches; a central axis is recognizable only near the apical cell; and an extensive cortex is formed (Kylin 1956).

Saito (1967) divided the genus *Laurencia* into two subgenera, *Laurencia* and *Chondrophycus* on the basis of a combination of the two features: 1) tetrasporangial arrangement (parallel to the fertile axis in *Laurencia* and right-angle in *Chondrophycus*) and 2) presence (*Laurencia*) or absence (*Chondrophycus*) of longitudinally oriented secondary pit-connections between contiguous superficial cortical cells. However, this treatment was later recognized to be impractical because of the occurrence of several species having the features of both subgenera. Nam & Saito (1995) found that the fundamental difference between two subgenera is not as above but the number of periaxial cells per vegetative axial segment: four in *Laurencia* and two in *Chondrophycus*. Garbary & Harper (1998) elevated the subgenus *Chondrophycus* to generic rank based on a cladistic analysis of the *Laurencia* complex.

Osmundea is delimited from the other two genera by its spermatangial development from apical and epidermal cells in the apical pit (filament type) rather than from trichoblasts derived from axial cells (trichoblast type) and by the production of tetrasporangia from random epidermal cells rather than from particular pericentral cells (Nam et al. 1994). The number of periaxial cells per vegetative axial segment is two in this genus (Nam et al. 1994).

The generic independency between *Laurencia* and *Chondrophycus* is supported by only one morphological feature. In the present study, we attempted to estimate the phylogenetic relationships within the *Laurencia* complex by molecular phylogenic analyses using the large subunit of RUBISCO (*rbc*L) gene sequences.

Materials and Methods

Specimens were collected from Ireland, Japan, Malaysia and the Philippines (Table 1). Total DNA was extracted from silica gel samples, or ethanol preserved specimens, or living materials, or culture strains, using a modified cetyltrimethyl ammonium bromide (CTAB) method (Kogame et al. 1999) or using the ISOPLANT II kit (Nippon Gene Co. Ltd., Toyama, Japan). After ethanol precipitation, the extracted DNA was purified using the GENECLEAN® II kit (Bio 101 Inc, Vista, Calif., USA). PCR amplification and cycle sequencing were conducted as in Kogame et al. (1999). Pairs of primer for PCR amplification and sequencing of the *rbc*L were used F8 with R643, F492c with R1138 and F945 with RH5 (Table 2). Phylogenetic analyses were performed

Table 1. Materials for DNA extraction.

Species	Locality (date)	
C. cartilagineus	Katsuura, Chiba, Japan (05.iii.2002)	living
C. concretus	Bolinao, Pangasinan, Philippines (23.xi.2004)	silicagel
C. intermedius	Katsuura, Chiba, Japan (05.iii.2002)	living
C. papillosus	Bolinao, Pangasinan, Philippines (23.xi.2004)	silicagel
C. parvipapillatus	Bolinao, Pangasinan, Philippines (23.xi.2004)	silicagel
C. undulatus	Choshi, Chiba, Japan (04.iii.2002)	living
C. sp. 1	Bolinao, Pangasinan, Philippines (23.xi.2004)	silicagel
C. sp. 2	Ogasawara Isl., Tokyo, Japan (v.2002)	living
C. sp. 3	Shimoda, Shizuoka, Japan	
L. brongniartii	Ishigaki Isl., Okinawa, Japan (30.i.2002)	ethanol
L. flexilis	Ishigaki Isl., Okinawa, Japan (30.i.2002)	ethanol
L. flexilis	Bolinao, Pangasinan, Philippines (23.xi.2004)	silicagel
L. hamata	Oga, Akita, Japan (23.ix.2000)	silicagel
L. intricata	Yonaguni Isl., Okinawa, Japan (07.v.2001)	silicagel
L. intricata	Bolinao, Pangasinan, Philippines (23.xi.2004)	silicagel
L. japonensis	Onahama, Fukushima, Japan	culture
L. lageniformis	Pulau Tikus, Sandakan, Sabah, Malaysia (14.v.1998)	culture
L. majuscula	Pulau Tikus, Sandakan, Sabah, Malaysia (14.v.1998)	culture
L. majuscula	Ishigaki Isl., Okinawa, Japan (30.i.2002)	ethanol
L. nipponica	Tanesashi, Hokkaido, Japan (24.vi.1989)	culture
L. okamurae	Inubozaki, Chiba, Japan	culture
L. okamurae	Hachijo Isl., Tokyo, Japan (21.vii.2001)	silicagel
L. omaezakiana	Tosashimizu, Kochi, Japan (06.vi.2001)	silicagel
L. omaezakiana	Hachijo Isl., Tokyo, Japan (20.vii.2001)	silicagel
L. pinnata	Heigun Isl., Yamaguchi, Japan (02.vi.1994)	culture
L. saitoi	Katsuura, Chiba, Japan (05.iii.2002)	living
L. similis	Pulau Sibu Besar, Johor, Malaysia (09.vi.1999)	culture
L. similis	Bolinao, Pangasinan, Philippines (23.xi.2004)	silicagel
L. tropica	Bolinao, Pangasinan, Philippines (23.xi.2004)	silicagel
L. venusta	Hinomisaki, Shimane, Japan (17.viii.1993)	culture
L. venusta	Amino, Kyoto, Japan (03.iv.1994)	culture
O. hybrida	Lugh Hyne, Cork County, Ireland (19.v.1993)	culture

Table 2. Oligonucleotide primers used for amplification and sequencing.

		Sequence (5′-3′)	Annealing site[1]
F8[2]	Forward	GGTGTAATTCCATACGCTAAAAATGGG	52–80
R643	Reverse	ATGGAAGCAGTAAATCGTTCAATT	676–699
F492c	Forward	ACGTATGGACAAATTTGGACG	492–512
R1138	Reverse	GTTGCTTCAGGTGGTATTCA	1138–1157
F945	Forward	GTGTTATTTGTAAATGGATGC	944–964
RH5[2]	Reverse	TAGAAACTCCAACAGCTTACGTTTAA	1442–1467

[1] Primer positions are indicated based on the DNA sequence of *rbc*L (*Antithamnion* sp., accession no. X54532).
[2] F8 and RH5 were referred to Shimada et al. (1999) and Yamagishi & Masuda (2000), respectively.

by maximum-likelihood (ML), implemented in PAUP* ver. 4.0b10 (Swofford 2002). The best-fit model of DNA substitution used in ML method was calculated by Modeltest 3.7 (Posada & Crandall 1998). The ML tree was found under the heuristic search method with 1000 random-addition-sequence replicates and TBR branch swapping. Bootstrap tests were run using the neighbor-joining (NJ) and the parsimony methods with the following options: 10000 replicates under Kimura-2-parameter distances (Kimura 1980) in NJ and 1000 bootstrap replication for each of 10 random-addition-sequence replicates with TBR branch swapping in parsimony. *Polysiphonia morrowii* Harvey (accession no. AY396030) and *Chondria dasyphylla* (Woodward) C. Agardh (accession no. U04021) were used as the outgroups. The following sequences were downloaded from the GenBank and used in our analyses: *Chondrophycus flagelliferus* (J. Agardh) Nam (AF465804); *Chondrophycus paniculatus* (C. Agardh) Furnari (AF489863) (as *Laurencia paniculata*); *Chondrophycus papillosus* (C. Agardh) Garbary et Harper [AF465807, AF489861 (as *Laurencia papillosa*)]; *Chondrophycus patentirameus* (Montagne) Nam (AF489862) (as *Laurencia patentiramea*); *Chondrophycus tronoi* (Ganzon-Fortes) Nam (as *Laurencia tronoi*) (AF489864); *Laurencia arbuscula* Sonder (AF465810); *Laurencia brongniartii* J. Agardh (AF465814); *Laurencia catarinensis* Cordeiro-Marino et Fujii (AF465808); *Laurencia complanata* (Suhr) Kützing (AF465813); *Laurencia flexilis* Setchell (AF489860); *Laurencia flexuosa* Kützing (AF465815); *Laurencia intricata* Lamouroux (AF465809); *Laurencia natalensis* Kylin (AF465816); *Laurencia obtusa* (Hudson) Lamouroux (AF465812); *Osmundea blinksii* (Hollenberg et Abbott) Nam (AY172575); *Osmundea hybrida* (de Candolle) Nam (AF281878); *Osmundea osmunda* (Gmelin) Nam et Maggs (AF281877); *Osmundea pinnatifida* (Hudson) Stackhouse (AF281876); *Osmundea ramosissima* (Oeder) Athanasiadis (AF281880); *Osmundea truncata* (Kützing) Nam et Maggs (AF281879); *Osmundea splendens* (Hollenberg) Nam (AY172576); *Osmundea spectabilis* var. *diegoensis* (Dawson) Nam (AY172572); *Osmundea spectabilis* var. *spectabilis* (Postels et Ruprecht) Nam (AY172574).

Results

The partial *rbc*L gene (1342 bp) sequences were successfully amplified and sequenced for 7 samples from the Philippines and 24 samples from Ireland, Japan and Malaysia, and corresponded to positions 96–1437 of the complete *rbc*L gene of *Antithamnion* sp. (accession no. AY396030). Figure 1 represents the ML tree (log likelihood=−10904.64291). This tree showed that 1) the genera *Osmundea* and *Laurencia* sensu stricto (except for *L. flexilis* and *L. tropica*) are monophyletic with high bootstrap values (100% in NJ/100% in parsimony); 2) the genus *Chondrophycus* is divided into two separate clades (referred to *Chondrophycus*-1 and *Chondrophycus*-2 in Fig. 1) and each clade has high bootstrap values (100% in NJ/100% in parsimony in the former, 100% in NJ/99% in parsimony in the latter). The *Chondrophycus*-1 clade includes the type species of *Chondrophycus*, *C. cartilagineus*; 3) the *L. tropica*/*L. flexilis* (AF489860) clade is most closely related to the *Chondrophycus*-2 clade and this cluster is moderately supported (94% in NJ/87% in parsimony); and 4) *L. flexilis* from Ishigaki Isl. (Japan) and the Philippines are firstly derived from the *Laurecia* sensu stricto lineage.

Discussion

Osmundea is morphologically distinguished from *Laurencia* and *Chondrophycus* by the filament-type spermatangial development and tetrasporangial production from random epidermal cells (Nam 1999). Generally such reproductive features are often regarded as good indicators of phylogenetic relationships. In this viewpoint, it is reasonable to treat *Osmundea* as an independent genus in the *Laurencia* complex. Our molecular phylogenetic result also supports this conclusion: the monophyly of the clade of *Osmundea* species was supported by high bootstrap values (Fig. 1).

All examined *Laurencia* species except for *L. flexilis* and *L. tropica* also clustered a well-supported clade (corresponding to the *Laurencia* sensu stricto, Fig. 1). The species in this clade seem to share the following features in common: four periaxial cells per vegetative axial segment; longitudinally oriented secondary pit-connections between contiguous superficial cortical cells; and *corps en cerise* within superficial cortical and trichoblast cells. On the other hand, *L. flexilis* and *L. tropica* constituted respective monophyletic clades with high bootstrap values[3]. *Laurencia flexilis* must be referred to the genus *Laurencia* redefined by Nam (1999) because of its four periaxial cells per vegetative axial segment (Masuda et al. 1999, fig. 31). However, this species is morphologically different from other typical *Laurencia* species in the absence of longitudinally oriented secondary pit-connections between contiguous superficial cortical cells (Masuda et al. 1999, fig. 33) and *corps en cerise* (unpublished observations). This suggests that genus *Laurencia* defined as the presence of four periaxial cells per vegetative axial segment is polyphyletic. As is well known, *corps en cerise* is thought to be the site of synthesis and/or storage of halogenated secondary metabolites (Young et al. 1980), which are considered to be effective against herbivores (Masuda et al. 1997). The presence or absence of *corps en cerise* may be one of phylogenetically critical features in the *Laurencia* complex.

[3] The identification of AF489860 seems to be doubtful.

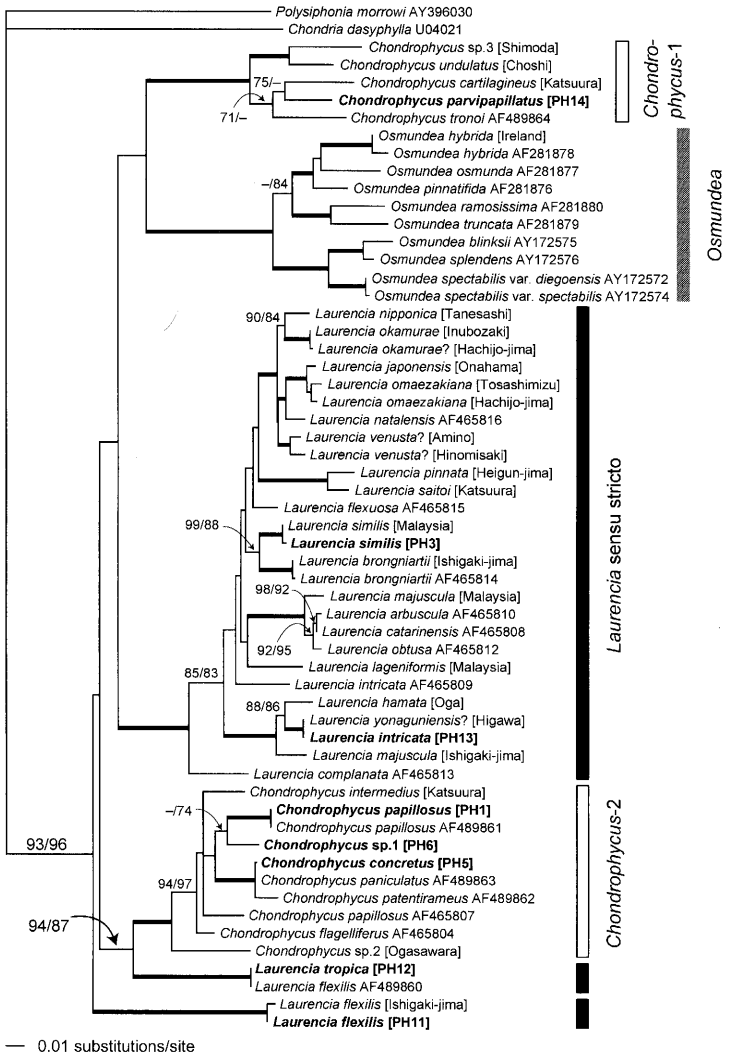

Fig. 1. Maximum-likelihood tree (log L = −10904.64291) of *Laurencia* sensu lato inferred from the partial *rbc*L sequences (1342 bp). Numbers on the nodes are bootstrap values, calculating from NJ (10000 replicates, left) and parsimony (1000 replicates with 10 random-sequence-addition, right). More than 70% of the values are shown. Names in brackets after species names indicate collection sites (see Table 1). Specimens from the Philippines are in bold.

Chondrophycus species were divided into two separated clades with high bootstrap values respectively (Fig. 1). One of them (*Chondrophycus*-1), which includes the generitype *C. cartilagineus*, weakly clustered with the *Osmundea* clade. The other one (*Chondrophycus*-2) is relatively highly supported to be monophyletic with *L. tropica*, which must be transferred to the genus *Chondrophycus* because of its two periaxial cells per vegetative axial segment (unpublished observations). These results suggest that *Chondrophycus* is polyphyletic and their two periaxial cells per vegetative axial segment may be evolved in parallel between the two *Chondrophycus* clades.

The branching order of the five clades, 1) *Osmundea*, 2) *Laurencia* with *corps en cerise*, 3) *L. flexilis*, 4) *Chondrophycus*-1 and 5) *Chondrophycus*-2/*L. tropica*, was not resolved because of low bootstrap values. The phylogenetic position of *L. flexilis*, a morphologically intermediate species between the typical *Laurencia* and *Chondrophycus*, must be clarified to determine the key features to distinguish *Chondrophycus* and *Laurencia* sensu stricto. Further investigations based on the other genes, for examples SSU or LSU rDNAs, are expected.

References

Garbary, D. J. and Harper, J. T. 1998. A phylogenetic analysis of the *Laurencia* complex (Rhodomelaceae) of the red algae. Cryptogamie, Algol., 19: 185–200.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol., 16: 111–120.

Kogame, K., Horiguchi, T. and Masuda, M. 1999. Phylogeny of the order Scytosiphonales (Phaeophyceae) based on DNA sequences of *rbc*L, partial *rbc*S and partial LSU nrDNA. Phycologia, 38: 496–502.

Kylin, H. 1956. Die Gattungen der Rhodophyceen. Gleerups, Lund.

Lamouroux, J. V. F. 1813. Essai sur les genres de la famille des Thalassiophytes non articulées. Ann. du Mus. d'Hist. Nat. Paris, 20: 21–47, 115–139, 267–293.

Masuda, M., Abe, T., Sato, S., Suzuki, T. and Suzuki, M. 1997. Diversity of halogenated secondary metabolites in the red alga *Laurencia nipponica* (Rhodomelaceae, Ceramiales). J. Phycol., 33: 196–208.

Masuda, M., Abe, T., Kawaguchi, S. and Phang, S. M. 1999. Taxonomic notes on marine algae from Malaysia I. Six species of Rhodophyceae. Bot. Mar., 42: 449–458.

Nam, K. W. 1999. Morphology of *Chondrophycus undulata* and *C. parvipapillata* and its implications for the taxonomy of the *Laurencia* (Ceramiales, Rhodophyta) complex. Eur. J. Phycol., 34: 455–468.

Nam, K. W. and Saito, Y. 1995. Vegetative and reproductive anatomy of some *Laurencia* (Ceramiales, Rhodophyta) species with a description of *L. maris-rubri* sp. nov. from the Red Sea. Phycologia, 34: 157–165.

Nam, K. W., Maggs, C. A. and Garbary, D. J. 1994. Resurrection of the genus *Osmundea* with an emendation of the generic delineation of *Laurencia* (Ceramiales, Rhodophyta). Phycologia, 33: 384–395.

Posada, D. and Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics, 14: 817–818.

Saito, Y. 1967. Studies on Japanese species of *Laurencia*, with special reference to their comparative morphology. Mem. Fac. Fish., Hokkaido Univ., 15: 1–81.

Shimada, S., Horiguchi, T. and Masuda, M. 1999. Phylogenetic affinities of genera *Acanthopeltis* and *Yatabella* (Gelidiales, Rhodophyta) inferred from molecular analyses. Phycologia, 38: 528–540.

Stackhouse, J. 1809. Tentamen marino-cryptogamicum. Mém. Soc. Imp. Nat. Moscou, 2: 50–97.

Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Uisng Parsimony (*and Other Methods). Version 4. Sinaur Associates, Sunderland, Massachusetts.

Yamagishi, Y. and Masuda, M. 2000. Taxonomic revision of a *Hypnea charoides-valentiae* complex (Rhodophyta, Gigartinales) in Japan, with a description of *Hypnea flexicaulis* sp. nov. Phycol. Res., 48: 27–35.

Young, D. N., Howard, B. M. and Fenical, W. 1980. Subcellular localization of brominated secondary metabolites in the red alga *Laurencia snyderae*. J. Phycol., 16: 182–185.