Applying the concept of metamorphosis to the crustose-to-erect thallus transition of macroalgae

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Synopsis Metamorphosis is broadly defined as a more or less radical morphological change between 2 multicellular life stages within an organism's life phase, often marking the transition from pre-reproductive to reproductive stages. It involves structural reorganization and major physiological changes, generally under the control of endogenous and exogenous factors and often resulting in changes in habitat use. This concept has been applied to the crustose-to-erect thallus transition of some red algae and the present study evaluates the validity of such hypotheses. Available literature suggests that the crustose-to-erect thallus transition involves significant morphological and habitat changes, separating pre-reproductive and reproductive stages. The onset of the morphological changes (for example axis differentiation) appears regulated by endogenous signals (growth factors) and growth is modified by environmental factors. The algae do not exhibit structural reorganization, however, probably due to their simple morphological structure and the lack of several cell and tissue mechanisms involving cell motility. The presence of cell walls in the algae impairs cell motility and maintains the cell in a fixed position within the plant. These are important differences restricting the extension of the definition of metamorphosis to the crust-to-erect thallus transition. The above restrictions also seem to apply to other macroalgae, fungi, and terrestrial plants.

Introduction

Life cycles and complex life-history transitions, including metamorphosis, often involve a large number of developmental processes that need to be accurately timed and coordinated with one another and with the environment (Heyland and others 2005). Since such processes have evolved many times in different environments (Hadfield 2000), complex life-history transitions are popular areas of study to advance evolutionary biology through comparative studies of convergent developmental processes.

Complex life-history transitions occur in a high diversity of organisms and involve a wide variety of processes. Accordingly, their study has stimulated such a wide range of approaches, theories, and methods that at present it is difficult to summarize the knowledge in this area due to a lack of common grounds among the organisms and processes studied (Heyland and others 2006). Consequently, efforts are being made to find similarities in life-history transitions among different kinds of organisms to provide the frame for a more unified approach to the study of these processes. A unified approach hopefully will improve our present understanding of these transitions, eventually opening up new avenues of research.

Present efforts to unify the concept of metamorphosis comprise a search for similarities and convergences in life-history transitions among distantly related organisms. Traditionally restricted to the Animalia, the concept has recently been expanded (Bishop and Hodin 2001; Hodin 2006) to include life-stage transitions in other non-animal multicellular eukaryotes, such as the mycelium-to-fruiting body transitions of some fungi and the crustose-to-thallus transition of some red algae (Heyland and others 2005).

The adoption of common terms to describe transitions between vegetative and reproductive multicellular stages of life histories across distantly related organisms and different kingdoms would facilitate comparisons of equivalent morphological and physiological responses, molecular regulators and signaling systems. Before expanding the definition in search for similarities, however, some critical assessment seems necessary. Such an assessment should evaluate whether or not applying a concept based on organisms in one Kingdom to organisms from another Kingdom would violate biological constraints in the latter. Even though each Kingdom of organisms (*sensu* Whittaker 1969; Margulis 1971) may include several kinds of organisms (Santelices 1999), each Kingdom has basic

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biological properties (Buss 1982, 1987) that might restrict the general application of definitions and concepts across all of them. In the present study we evaluate this idea by applying the expanded concept of metamorphosis to the crustose-to-erect thallus transition of some red algal species. We start with a broad definition of metamorphosis. Then we evaluate the applicability of each component of this definition to the crustose-to-erect thallus transition. Finally, we discuss whether the concept could be applied to organisms from non-animal kingdoms.

Definition of metamorphosis

Metamorphosis is understood (Hodin 2006) as a more or less radical morphological change between 2 multicellular phases in an organism's life cycle, often marking the transition from a pre-reproductive to a reproductive life stage. It involves structural reorganization and major physiological and habitat changes and is controlled by endogenous and exogenous factors.

The crustose-to-erect thallus transition in the red algae

Algal species in the Orders Corallinales, Hildenbrandiales, Palmariales, Gigartinales and Rhodymeniales all exhibit crustose-to-erect thallus transitions. After attachment, the spore divides by a wall perpendicular to the substratum. Several vertical and horizontal divisions follow, producing a hemispherical, polystromatic crust (Dixon 1973; Gabrielson and Garbary 1986). Fifteen to thirty days after spore germination, one or a group of transversely dividing axial cells arise from within or from the surface of the crust, producing a number of axial filaments (Figs 1A and B). Axial filaments are often surrounded by lateral filaments that also exhibit apical divisions and increase the width of the emerging upright. The degree of aggregation between lateral and axial filaments varies among species, but in species with erect, foliose thalli there is a rather compact arrangement.

The continued activity of axial and lateral apicals eventually leads to the formation of a macroscopically recognizable erect thallus arising from the crustose base (Fig. 1C). Thus, the crustose-to-erect thallus transition occurs during development of the gametophyte, the sporophyte, or both. The transition does not refer to interphase changes, for example from a crustose gametophyte to a foliose sporophyte, but it is restricted to intra-phase changes.

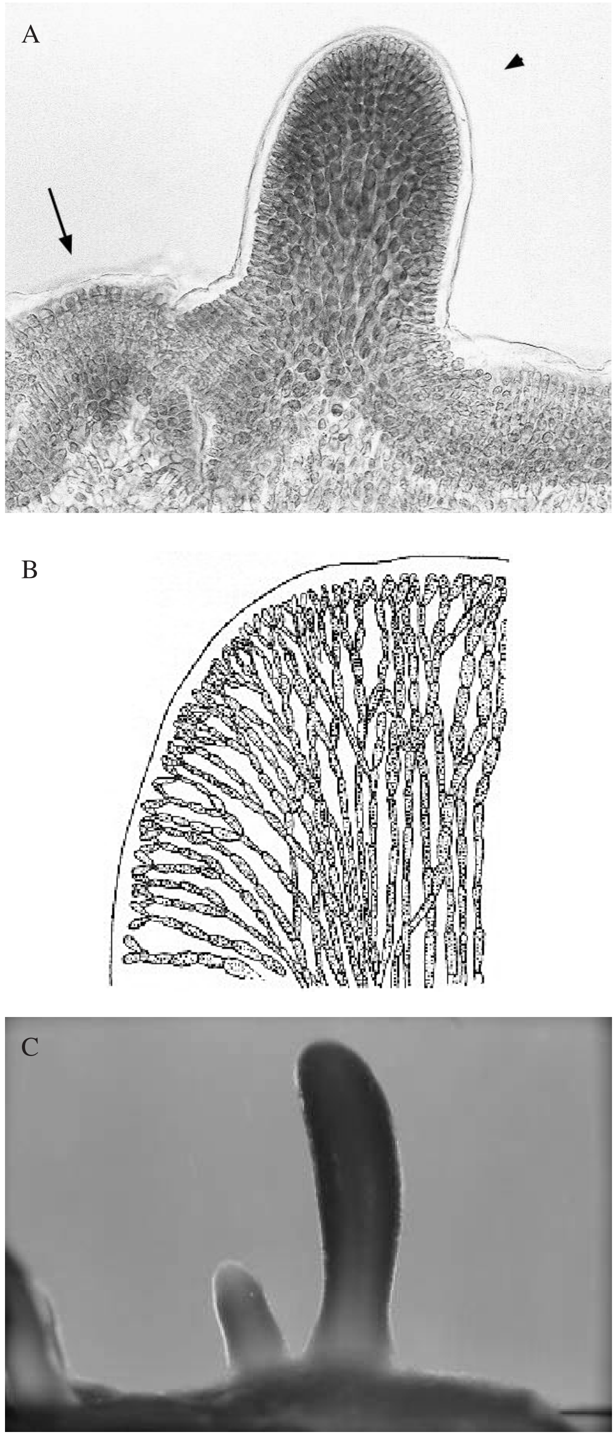

Fig. 1 The crustose-to-erect thallus transition in red algae. (**A**) Differentiation (arrow) of a group of transversely dividing axial cells starting to emerge above the crust surface. Note the larger size of the apical cells. A larger axis (arrow head) is already extending in the vertical direction. Note the apical and lateral initials. (**B**) Diagram showing cellular differentiation and growth during the early uprising of an erect axis. (**C**) Two erect axes of different ages arising from a common basal crust.

Table 1 Evaluation of the applicability of concepts used in the definition of metamorphosis to the crustose-to-erect thallus transition of red algae

Concepts	Yes	No
Significant morphological change occurring between 2 multicellular life stages within a life phase	√	
Involving physiological changes	√	
Involving habitat changes	√	
Controlled by endogenous factors	√	
Affected by exogenous factors	√	
Making a transition from pre-reproductive to reproductive stages	√	
Involving structural reorganization		√

Table 2 Examples of endogenous regulation of thallus uprising in red algae

Species	Compound	Author
Gracilaria tenuistipitata and *Gracilaria perplexa*	Indole-3-acetic acid (IAA) Dichlorophenoxyacetic acid (2,4 D)	Yokoya and colleagues (2004)
Solieria filiformis	Cytokinins (K)	Yokoya and colleagues (2004)
Grateloupia doryphora	Gibberelic acid (GA)	García-Jiménez and colleagues (1998)

Application of the conceptual components of metamorphic changes to the crustose-to-erect thallus transition of the red algae

Available literature is used to evaluate the application of the conceptual components of the above definition of metamorphosis to the crustose-to-erect thallus transition of the red algae (Table 1). All but one of the concepts are fulfilled by the algal life-stage transition.

Within the morphological simplicity of the red algal thallus, the morphological changes involved in the emergence of the erect axes are significant. The process involves the differentiation of a number of axial cells that, by transverse divisions, produce filaments in a vertical direction, rising well above the level of the crust and originating a new erect axis. The net effect of this is a change in the direction of the main growth, from radial expansion of the crust to a vertical extension of the axis. In addition, the differentiation of axial and lateral filaments, both with apical initials, allows the increments in length and breadth of the erect axes.

The emergence and growth of the erect axis also implies a change of habitats. The growing thallus extends from the boundary layer and laminar sublayer habitats to places with higher water velocities, such as the turbulent sublayers (Neushul 1972; Charters and others 1973; Denny 1988; Lobban and Harrison 1994). It is generally known that water velocity modifies the net effects of several other abiotic factors on the growth of macroalgae (see review in Lobban and Harrison 1994), including irradiance, speed of nutrient exchange, sediment accumulation on the thallus, and epiphyte load. Therefore, even though the crustose-to-erect thallus transition in these red algal species does not involve a spatial shift, it does imply changes in the habitat conditions affecting the seaweed and changes in the physiological responses of the growing axes.

Evidence for endogenous and exogenous regulation of thallus uprising has been obtained, among others, through tissue cultures. The addition of different concentrations of several types of growth regulators to calluses of various algal species (see Table 2) results in a differentiation of apical initials and the growth of the erect axis. Studies on evolutionary patterns in auxin action (Cooke and others 2002) have concluded that the mechanisms mediating the responses of some of these hormones (for example indole-3-acetic acid) in land plants already existed in algae and bryophytes.

The crustose-to-erect thallus transition can also be modified by environmental factors such as temperature, irradiance, and salinity. Although numerous studies (see Lobban and Harrison 1994 for review) have evaluated the effects of these factors on the growing axes under controlled laboratory conditions, only a few (for example Yokoya and others 1999) have worked with uprisings differentiated from calluses in tissue culture.

The crustose-to-erect thallus transition also separates pre-reproductive and post-reproductive stages in most of these algae. With a few exceptions (for example some species in the Corallinales), most taxa with erect axes generally differentiate reproductive structures only on those axes that, by later growth, become foliose, with morphologies ranging from cylindrical axes to expanded blades (see Wommersley 1996; Stegenga and others 1997 or Abbott 1999 for examples and descriptions).

Several organismic factors may also modify the number and timing of erect axis arising from basal crusts, especially among coalescing species. These include the number of spores coalescing during early recruitment (Santelices and others 1996, 1999) and the number of young recruits coalescing with older ones (Santelices and others 2004). Equivalent factors, however, have not been included in the definition of metamorphosis, probably because they do not occur or analogous responses are not found in metamorphic animals.

The crustose-to-thallus transition of the red algae does not involve structural remodeling as the metamorphic changes of animals do. Repeated observations of the uprising process (for example Fig. 1) only indicate cell and tissue proliferation, without evidence of cell or tissue transfers within the plant, as animals do during metamorphosis. Since this is the only component of metamorphic change not found in the algal life-stage transition under study, some evaluation of its importance seems necessary.

Evaluation of differences

When exploring causes for the lack of structural remodeling in the life-stage transitions of the algae, 3 factors of general importance emerge. The first refers to the very simple body (thallus) structure of the algae, in comparison with animals. While in most animals, a diversity of tissues and specialized cells can be found, the crustose and erect axis of the algae have a relatively small variety of cells and tissues (cortex and medulla) conforming the structure of the growing axis.

A second important difference refers to the cellular and tissue mechanisms involved in metamorphosis in animals and their presence in macroalgae. While there are 6 potential processes in animals (Table 3), only cell and tissue proliferation are of common occurrence in macroalgae. At present, there is not enough information to know whether apoptosis occurs in red algae but it is clear that these organisms lack cell migration and tissue resorption. The presence of cell walls in algae impairs cell motility (Buss 1982, 1987), maintaining the cell in a fixed position within the plant. In turn, the lack of motility impairs cell migration and tissue resorption. In fact, macroalgae may move nutrients and organic substances from one part of the plant to another (see Lobban and Harrison 1994; Gonen and others 1996) but cannot reabsorb tissues. Cell fusions have been described in a few red macroalgal species during cystocarp formation (Gabrielson and Garbary 1986) or during spore coalescence (Santelices and others 1999) before cell walls are differentiated, but in general cell wall formation limits the capacity to fuse in these cells. Contiguous algal cells may establish cellular connections but they cannot fuse.

The third general difference found between the metamorphic changes in most animals and the crustose-to-thallus transition of the red algae occurs at the individual level. During typical metamorphosis of animals, the whole individual of the previous metamorphic stage (for example a larva) transforms itself into the next metamorphic stage (for example a pupae). In contrast, in the crustose-to-erect thallus transition of the macroalgae, the crust (previous metamorphic stage) does not transform itself into the new, erect axis (new metamorphic stage). The crust remains there, normally growing radially and eventually originating additional erect axes. In this process the crustose-to-erect thallus transition of the red algae approaches the budding off medusae of scyphozoan and hydrozoan polyps rather than exhibiting a typical metamorphic transformation. A crustose-to-thallus transition also could be found in other types of macroalgae (for example *Analipus*, a brown alga) but it is expected that they exhibit the above differences described for the red algae. Similarly, several of the above differences probably occur also in fungi and land plants, the other examples of non-animal metamorphosis suggested (Bishop and Hodin 2001; Heyland and others 2005), because these organisms also exhibit cell walls (Buss 1982, 1987).

Table 3 Cellular and tissue mechanisms involved in the metamorphosis of Animalia and their presence in red algae

Component	Drosophila[1]	Tunicates[2]	Amphibians[3]	Red algae
Cell proliferation	√	√	√	√
Cell migration	√	√	√	–
Apoptosis	√	√	√	?
Tissue proliferation	√	√	√	√
Tissue resorption	√	√	√	–
Tissue fusion	√	√	√	In a few stages

[1]*Drosophila:* Deng and colleagues (1999), Ishimaru and colleagues (2004), Fernandes and Keshishian (2005), Guha and Kornberg (2005), Kilpatrick and colleagues (2005), Waldhuber and colleagues (2005), Yin and Thummel (2005).
[2]Tunicates: Sato and colleagues (1997), Davidson and Swalla (2002), Bates (2004), Tarallo and Sordino (2004), Barenbrock and Kock (2005), Weill and colleagues (2005).
[3]Amphibians: Olson and Hanken (1996), Ishizuya-Oka and colleagues (2000), Gianola and colleagues (2001), Fu and colleagues (2005), Ishizuya-Oka and Shi (2005), Rowe and colleagues (2005), Schreiber and colleagues (2005), Wagner and Helbing (2005).

Conclusion

It is concluded that the crustose-to-erect thallus transition of the red algae parallels many of the responses so far described for the metamorphic processes of Animalia. However, the algae exhibit important biological restrictions due to the presence of a cell wall, lack of cell motility, a very simple morphological structure, and the persistence of the later metamorphic stages after the emergence of the new metamorphic stage. Several of these constraints are also anticipated to occur in other kinds of macroalgae, fungi, and land plants.

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References

Abbott IA. 1999. Marine red algae of the Hawaiian Islands. Honolulu, Hawaii: Bishop Museum Press.

Barenbrock JS, Kock M. 2005. Screening enzyme-inhibitory activity in several ascidian species from Orkney Islands using protein tyrosine kinase (PTK) bioassay-guided fractionation. J Biotechnol 117:225–32.

Bates WR. 2004. Cellular features of an apoptotic form of programmed cell death during the development of the ascidian, *Boltenia villosa*. Zool Sci 21:553–63.

Bishop C, Hodin J. 2001. Metamorphosis. Dev Biol 235:242.

Buss LW. 1982. Somatic cell parasitism and the evolution of somatic tissue compatibility. Proc Natl Acad Sci USA 79:5337–41.

Buss LW. 1987. The evolution of individuality. Princeton, NJ: Princeton University Press.

Charters AC, Neushul M, Coon D. 1973. The functional morphology of *Eisenia arborea*. Proc Int Seaweed Symp 5:29–54.

Cooke TJ, Poli D, Sztein AE, Cohen JD. 2002. Evolutionary patterns in auxin action. Plant Mol Biol 49:319–38.

Davidson B, Swalla BJ. 2002. A molecular analysis of ascidian metamorphosis reveals activation of an innate inmune response. Development 129:4739–51.

Deng WM, Leaper K, Bownes M. 1999. A targeted gene silencing technique shows that *Drosophila* myosin VI is required for egg chamber and imaginal disc morphogenesis. J Cell Sci 112:3677–90.

Denny MW. 1988. Biology and the mechanics of the wave-swept environment. Princeton, NJ: Princeton University Press.

Dixon PS. 1973. Biology of the Rhodophyta. Koenigstein, Germany: Otto Koeltz Science Publishers.

Fernandes JJ, Keshishian H. 2005. Motoneurons regulate proliferation and patterning in *Drosophila*. Dev Biol 277:493–505.

Fu LZ, Ishizuya-Oka A, Buchholz DR, Amano T, Matsuda H, Shi YB. 2005. A causative role of stromelysin-3 in extracellular matrix remodeling and epithelial apoptosis during intestinal matamorphosis in *Xenopus laevis*. J Biol Chem 280:27856–65.

Gabrielson PW, Garbary D. 1986. Systematics of red algae (Rhodophyta). CRC Crit Rev Plant Sci 3:325–66.

García-Jiménez P, Rodrigo M, Robaina RR. 1998. Influence of plant growth regulators, polyamines and glycerol interaction on growth and morphogenesis of carposporelings of *Grateloupia* cultured in vitro. J Appl Phycol 10:95–100.

Gianola S, Clairambault P, Franzoni MF. 2001. Relationships between neuronal cell adhesion molecule and LHRH neurons in the urodele brain: a developmental immunohistochemical study. Eur J Histochem 45:229–38.

Gonen Y, Kimmel E, Tel-Or E, Friedlander M. 1996. Intercellular assimilate translocation in *Gracilaria cornea* (Gracilariaceae, Rhodophyta). Hydrobiologia 326/327:421–8.

Guha A, Kornberg TB. 2005. Tracheal branch repopulation precedes induction of the *Drosophila* dorsal air sac primordium. Dev Biol 287:192–200.

Hadfield MG. 2000. Why and how marine-invertebrate larvae metamorphose so fast. Semin Cell Dev Biol 11:437–43.

Heyland A, Bishop C, Hodin J, Moroz L. 2006. Synopsis. (This symposium).

Heyland A, Hodin J, Reitzel AM. 2005. Hormone signaling in evolution and development: a non-model system approach. Bioessays 27:64–75.

Hodin J. 2006. Expanding networks: a hypothesis for the evolution of metamorphosis. (This symposium).

Ishimaru S, Ueda R, Hinohara Y, Ohtani M, Hanafusa H. 2004. PVR plays a critical role via JNK activation in thorax closure during *Drosophila* metamorphosis. EMBO J 23:3984–94.

Ishizuya-Oka A, Shi YB. 2005. Molecular mechanisms for thyroid hormone-induced remodeling in the amphibian digestive tract: a model for studying organ regeneration. Dev Growth Differ 47:601–7.

Ishizuya-Oka A, Li Q, Amano T, Damjanovski S, Ueda S, Shi YB. 2000. Requirement for matrix metalloproteinase stromelysin-3 in cell migration and apoptosis during tissue remodeling in *Xenopus laevis*. J Cell Biol 150:1177–88.

Kilpatrick ZE, Cakourus D, Kuman S. 2005. Ecdysone-mediated up-regulation of the effector caspase DRICE is required for hormone-dependent apoptosis in *Drosophila* cells. J Biol Chem 280:11981–6.

Lobban CS, Harrison PJ. 1994. Seaweed ecology and physiology. Cambridge, USA: Cambridge University Press.

Margulis L. 1971. Whittaker-five kingdoms of organisms: minor revisions suggested by considerations of the origin of mitosis. Evolution 25:242–5.

Neushul M. 1972. Functional interpretation of benthic marine algal morphology. In: Abbot IA, Kurogi M, editors. Contributions to the systematics of benthic marine algae of the North Pacific. Tokyo: Japan Society for Phycology. p 47–74.

Olsson L, Hanken J. 1996. Cranial neural-crest migration and chondrogenic fate in the oriental fire-bellied toad *Bombina orientalis*: defining the ancestral pattern of head development in anuran amphibians. J Morphol 229:105–20.

Rowe I, Le Blay K, Du Pasquier D, Palmier K, Levi G, Demeneix B, Coen L. 2005. Apoptosis of tail muscle during amphibian metamorphosis involves a caspase 9-dependent mechanism. Dev Dyn 233:76–87.

Santelices B. 1999. How many kinds of organisms are there? Trends Ecol Evol 14:152–5.

Santelices B, Correa J, Aedo D, Hormazabal M, Flores V, Sanchez P. 1999. Convergent biological processes among coalescing Rhodophyta. J Phycol 35:1127–49.

Santelices B, Correa JA, Meneses I, Aedo D, Varela D. 1996. Sporeling coalescence and intraclonal variation in *Gracilaria chilensis* (Gracilariales, Rhodophyta). J Phycol 32:313–22.

Santelices B, Hormazabal M, Correa J, Varela D. 2004. The fate of overgrown germlings in coalescing Rhodophyta. Phycologia 43:346–52.

Sato Y, Terakado K, Morisawa M. 1997. Test cell migration and tunic formation during posthatching development of the larva of the ascidian, *Ciona intestinalis*. Dev Growth Differ 39:117–26.

Schreiber AM, Cai LQ, Brown DD. 2005. Remodeling of the intestine during metamorphosis of *Xenopus laevis*. Proc Natl Acad Sci U S A 102:3720–5.

Stegenga H, Bolton JJ, Anderson RJ. 1997. Seaweeds of the South African West Coast. Contributions from the Bolus Herbarium Number 18. Cape Town, South Africa: Creda Press.

Tarallo R, Sordino P. 2004. Time course of programmed cell death in *Ciona intestinalis* in relation to mitotic activity and MAPK signaling. Dev Dyn 230:251–62.

Wagner MJ, Helbing CC. 2005. Multiple variants of the ING1 and ING2 tumor suppressors are differentially expressed and thyroid hormone-responsive in *Xenopus laevis*. Gen Comp Endocrinol 144:38–50.

Waldhuber M, Emoto K, Petritsch C. 2005. The *Drosophila* caspase DRONC is required for metamorphosis and cell death in response to irradiation and developmental signals. Mech Develop 122:914–27.

Weill M, Philips A, Chourrout D, Fort P. 2005. The caspase family in urochordates: distinct evolutionary fates in ascidians and larvaceans. Biol Cell 97:857–66.

Whittaker RH. 1969. New concepts of kingdoms of organisms. Science 163:150–60.

Womersley HBS. 1996. The Marine benthic flora of Southern Australia. Rhodophyta. Part IIIB, Gracilariales, Rhodymeniales, Corallinales and Bonnemaisoniales. Flora of Australia Supplementary Series Number 5. Canberra, Australia: Australian Biological Resources Study.

Yin VP, Thummel CS. 2005. Mechanisms of steroid-triggered programmed cell death in *Drosophila*. Semin Cell Dev Biol 16:237–43.

Yokoya NS, Kakita H, Obika H, Kitamura T. 1999. Effects of environmental factors and plant growth regulators on growth of the red alga *Gracilaria vermiculophylla* from Shikoku Island, Japan. Hydrobiologia 398/399:339–47.

Yokoya NS, West JA, Luchi AE. 2004. Effects of plant growth regulators on callus formation, growth and regeneration in axenic tissue cultures of *Gracilaria tenuistipitata* and *Gracilaria perplexa* (Gracilariales, Rhodophyta). Phycological Res 52:244–54.