3. Velayudhan, K. C., Muralidharan, V. K. and Amalaraj, V. A., *J. Econ. Tax. Bot.*, 1990, **14**, 579–582.
4. Baruah, P. and Sarma, G. C., *ibid*, 1987, **11**, 71–76.
5. McCarron, M., Mills, A. J., Whittaker, D., Sunny, T. P. and Verghese, J., *Flavour Fragrance J.*, 1995, **10**, 355–357.
6. Zwaving, J. H. and Bos, R., *ibid*, 1992, **7**, 19–22.
7. Dung, N. X., Tuyet, N. T. B. and Leclercq, P. A., *J. Essent. Oil Res.*, 1995, **7**, 701–703.
8. Ramachandraiah, O. S., Azeemoddin, G. and Krishnama Charyulu, J., *Indian Perfum.*, 1998, **42**, 124–127.
9. Choudhury, S. N., Ghosh, A. C., Saikia, M., Choudhury, M. and Leclercq, P. A., *J. Essent. Oil Res.*, 1996, **8**, 633–638.
10. Sharma, R. K., Misra, B. P., Sarma, T. C., Bordoloe, A. K., Pathak, M. G. and Leclerq, P. A., *ibid*, 1997, **9**, 589–592.
11. Verma, V. P. S., Shiva, M. P., Suki, R. K. and Subramanyan, I. V., *Indian For.*, 1978, **104**, 846.
12. Panda, R. and Panda, H., *ibid*, 1987, **113**, 434–440.
13. Dung, N. X., Truong, P. X., Ky, T. Pham and Leclerq, P. A., *J. Essent. Oil Res.*, 1997, **9**, 677–681.

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Porphyra – the economic seaweed as a new experimental system

Marine algae, popularly known as seaweeds, are sources of food, fodder, fertilizer, medicine and chemicals[1]. World trade in seaweed and its products was valued at US $ 50 million in 1970, US $ 250 million in 1990 and US $ 6.2 billion in 1999 (refs 1 and 2). About 20,000 marine algae species are distributed throughout the world, out of which only 221 species are utilized commercially. These include 145 species for food and 110 species for phycocolloid production[1]. *Porphyra* (Bangiales, Rhodophyta) popularly known as 'Nori' in Japan, 'Kim' in Korea and 'Zicai' in China has an annual value of over US $ 1.8 billion[3]. *Porphyra* is primarily used as food, wrapped around the Japanese delicacy 'Sushi' which consists of roasted blades, raw fish, rice and other ingredients. The alga is not only delicious but also contains high levels of protein (25–50%), vitamins (higher vitamin C than in oranges), trace minerals and dietary fibres[4]. The plant contains nearly 17 types of free amino acids, including taurine which controls blood cholesterol levels[5]. The alga is a preferred source of the red pigment *r*-phycoerythrin, which is utilized as a fluorescent 'tag' in the medical diagnostic industry[6]. *Porphyra* has been cultivated for the past hundred years in Japan and today it is one of the largest aquaculture industries in Japan, Korea and China[6]. Because of its economic importance and other health benefits, *Porphyra* cultivation is now being expanded to other countries[7]. Recently, it has been found that the plant has much more potential and can be used as an experimental system like *Arabidopsis thaliana* in the higher plants, some aspects of which are discussed here.

Nearly 133 species of *Porphyra* have been reported from all over the world, which includes 28 species from Japan, 30 from North Atlantic coasts of Europe and America and 27 species from the Pacific coast of Canada and United States[8]. Although seven species have been reported from the Indian coast, these are not being exploited commercially[9]. The genus *Porphyra* has a simple morphology. The plants are either round, round to ovate, obovate, linear or linear lanceolate (Figure 1 *a–d*). Individuals can also have blades that may be divided into male and female sections[10], or have a sectored morphology[11]. The plants can grow from 5 to 35 cm in length. The thalli are either one or two cells thick, and each cell has one or two stellate chloroplasts with a pyrenoid.

Porphyra has a heteromorphic life cycle with an alternation between a macroscopic foliose thallus which is the gametophytic phase, and a filamentous sporophyte called conchocelis phase. This diploid conchocelis phase in the life cycle was earlier thought to be *Conchocelis rosea*, a shell-boring organism. However, it was Drew in 1949 who demonstrated in culture that *P. umbilicalis* (L.) Kütz had a diploid conchocelis phase[12]. Until this landmark work concholeis was considered as an independent organism. These findings completely revolutionized the *Porphyra* industry in Japan and subsequently throughout Asia.

Porphyra reproduces by both sexual and asexual modes of reproduction. In sexual reproduction, certain mature vegetative cells of the thallus get differentiated into carpogonia, and others on the same or different thallus get differentiated into colourless spermatangia. After fertilization, the carpogonia divides to form packets of spores called zygotospores (carpospores). After release, the zygotospores usually germinate unipolarly to produce the filamentous conchocelis phase. The conchocelis can survive in adverse environmental conditions, but give rise to conchosporangia and conchospores under suitable conditions. The conchospores germinate by bipolar modes to give rise to young chimeric thalli, thus completing the life cycle (Figure 2).

In asexual reproduction, the vegetative cells in some species directly form the spores called archeospores which can directly germinate to form the thallus[13] (Figure 1 *i*). Recently, it has been found that besides these two modes of reproduction, *Porphyra* also reproduces by endosporangia or endospores which ultimately give rise to the thallus[14].

Different stages in the life cycle can be manipulated both in the laboratory as well as on an industrial scale. Large-scale conchocelis are being cultured in suitable environmental conditions by different Nori companies. Massive amount of con-

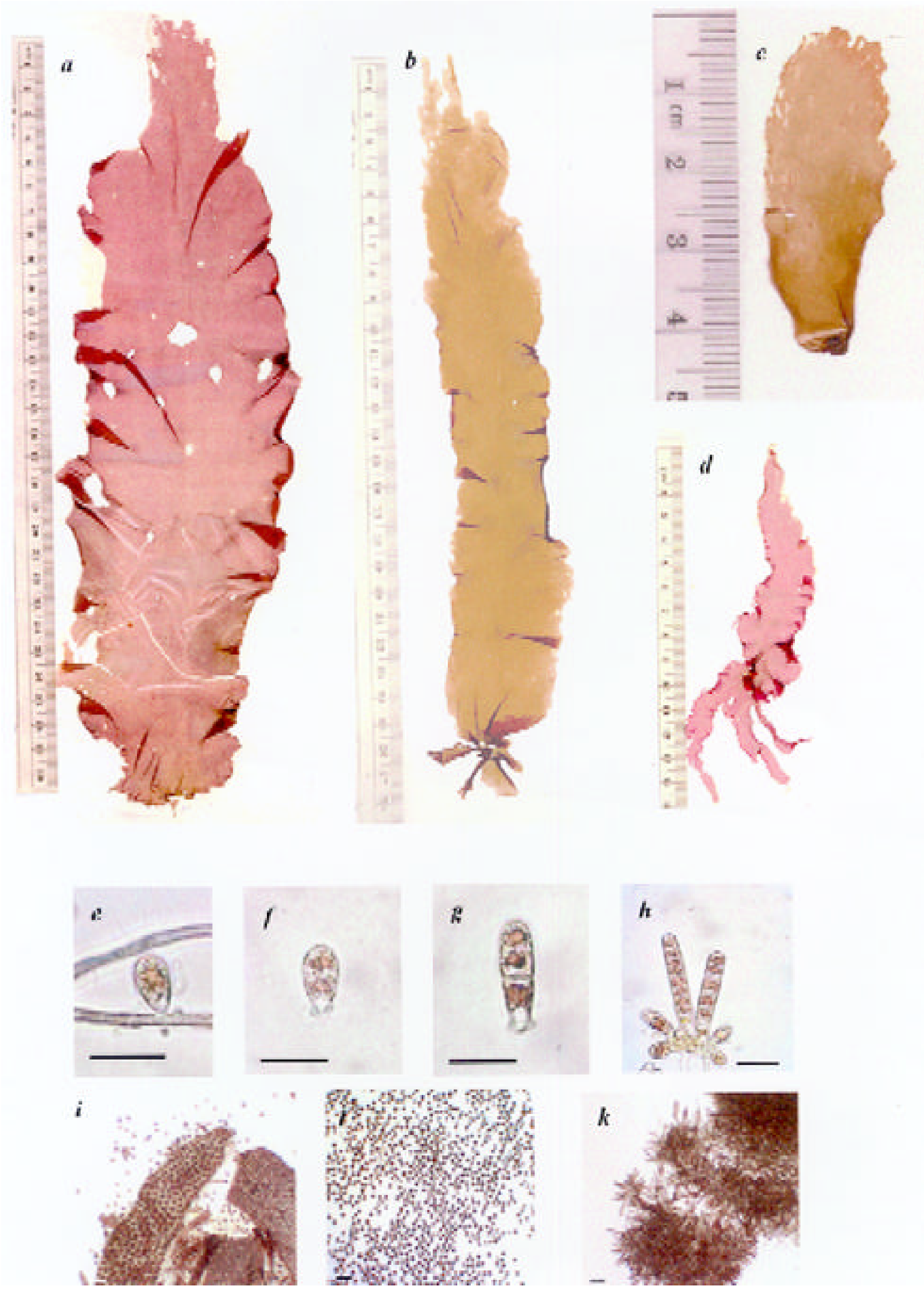

Figure 1. ***a–k***. *Porphyra* sp. Morphology and development. ***a***, Field material of *Porphyra kanyakumariensis* from Kerala, India; ***b***, Cultured *P*. *katadai* var. *hemiphylla* from Qingdao, P.R. China; ***c***, Field material of *P*. *yezoensis* from Qingdao, P.R. China; ***d***, Field material of *P*. *vietnamensis* from Goa, India; ***e–h***, Different stages of conchospore development, Scale bars = 25 µm; ***i***, Release of archeospores from the mature thallus. Several young thalli were formed from these spores. Scale bar = 50 µm; ***j***, Massive conchospores released from conchosporangia. Scale bar = 25 µm; and ***k***, Large number of young and homogenous thalli in culture after the germination of conchospores. Scale bar = 100 µm.

chospores can be released (Figure 1 *j*), seeded onto the nori nets and subsequently can be transplanted into the sea for large-scale cultivation. Traditionally, a large amount of shells ($1–2 \times 10^6$ shells) are required to maintain and grow conchocelis on a commercial scale. This occupies an enormous amount of space and the methods are labour-intensive. Recent research has shown that free conchocelis can be maintained in a large-scale inside bioreactors.

Although several studies have been made on different aspects of *Porphyra*, light, temperature and photoperiod play a major role by which its life cycle can be manipulated.

It has been found that temperature and photoperiod play a major role in each stage of the life history of *Porphyra*. For example, in *P. dentata* and *P. pseudolinearis*, the growth and maturation of conchocelis occurred between 10 and 25°C. Interestingly, conchospore liberation does not take place under these culture conditions. However, when the conchosporangia are transferred from 20 to 15°C, the conchospores get released. While spermatia get released at 10 and 15°C, zygotospores get liberated only at 15°C (ref. 15). In *P. leucosticta* the growth of conchocelis was twice as fast at 15°C in short-day condition compared to the day-neutral and long-day condition, whereas the gametophytic thalli grew well at 15°C in short-day condition. When conchocelis were transferred from the high light intensity to low light intensity, conchosporangia were found in 1–2 weeks, but conchospores did not liberate up to one month. The life cycle was completed in the laboratory in 2–3 months[16]. In a similar study in *P. leucostica* from USA, the present authors found that the life cycle can be completed in less than one month at 15°C 12:12 L:D conditions (unpublished). This suggests that the same species can have different behaviour under different environmental conditions. A number of studies reveal that each stage in the life cycle of *Porphyra* is species-specific and dependent on light, temperature and photoperiod[17–20].

An emerging problem of coastal mariculture activities is the significant loading of inorganic nutrients into local waters. Nutrient enrichment from fish and shrimp farming is equivalent to that of municipal sewage. So it is important to remove the excessive nitrogen and phosphorus from the aquaculture systems in a sustainable way. Recently, it has been found that different species of *Porphyra* can be effectively used as nutrient scrubbers[21,22]. Because of its rapid nutrient-removal capacity from the environment, *Porphyra* is now being planned for use in integrated finfish culture[23].

As mentioned earlier, *Porphyra* produces three different kinds of spores: archeospores, zygotospores and conchospores. The archeospores are haploid,

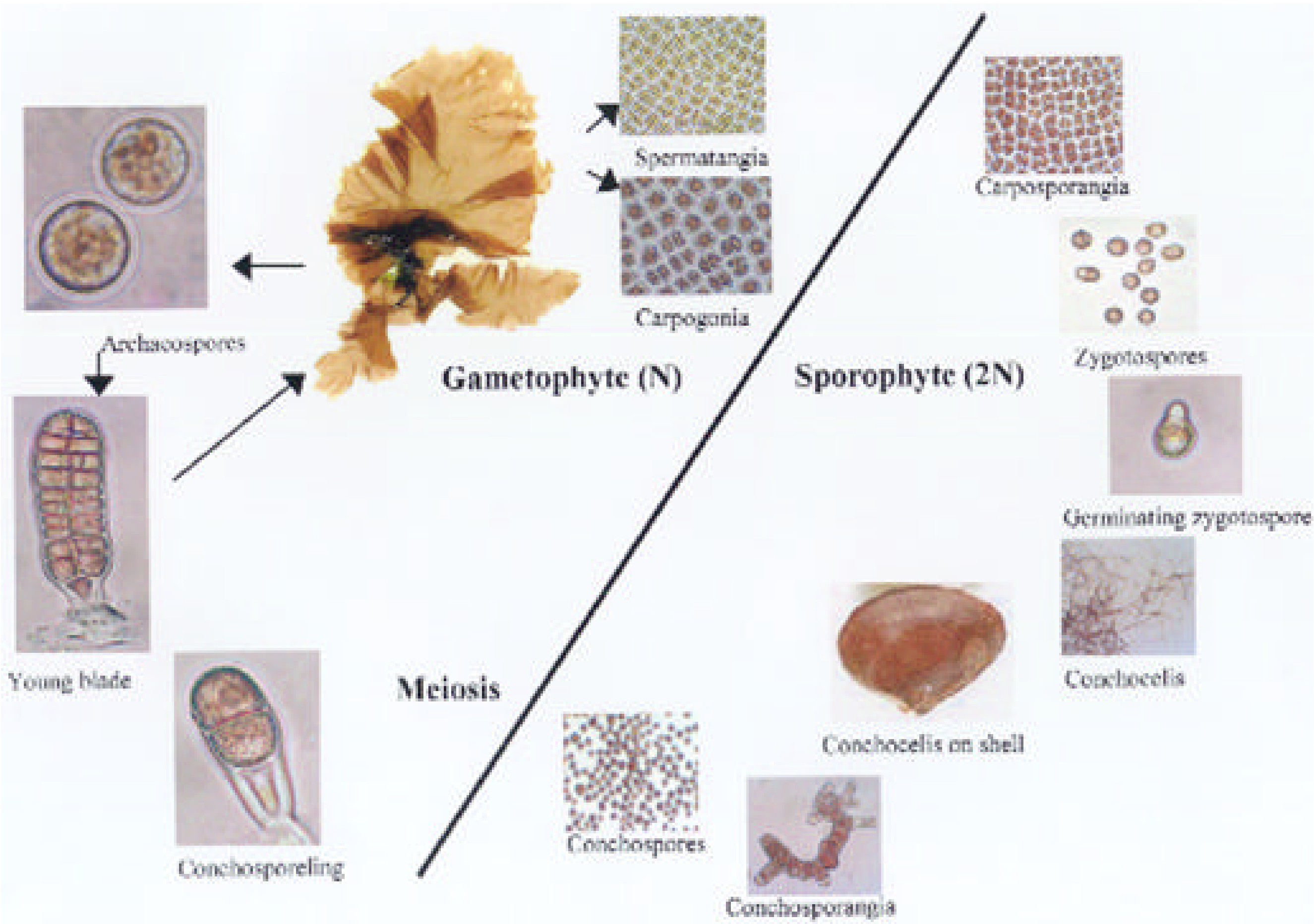

Figure 2. Life history of *Porphyra* showing different stages of development.

directly germinate and subsequently divide to give rise to the thallus (Figure 1 *i*). In contrast, the zygotospores and conchospores are diploid, while zygotospores give rise to conchocelis (sporophyte), the conchospores undergo meiosis to give rise to haploid thalli that may be considered chimeric individuals[11] (Figure 1 *g*, *h*). One can easily study each stage of development under a microscope without much difficulty (Figure 1 *e*–*h*). Since the thallus is either single-layered or double-layered, it is a good material for cytological studies. One can see 2–5 pairs of chromosomes at different stages of mitosis and meiosis[24,25]. Protoplast isolation and fusion amongst various species of *Porphyra* have been achieved, including intergeneric somatic hybridization[26–28]. Morphogenetic studies have also been carried out from the *Porphyra* protoplast[29].

Porphyra has recently been gaining momentum as a model plant for basic and applied studies in plant science. There are several advantages to study development, physiology, cytology, genetics and genomics. For example, establishment of several pure lines (Figure 1 *k*), the small genome size which is estimated to be 2.6×10^8 base pairs consisting of three chromosomes and short generation time (1–3 months) are suitable for genetic analysis. To understand the whole genetic system in red algae, large-scale expressed sequence tag (EST) analysis of *P. yezoensis* has been initiated. Normalized and size-selected cDNA libraries were generated from both gametophytic and sporophytic stages of *P. yezoensis* Ueda (strain TU-1), and single-pass sequencing was performed from the 5′-end of each cDNA clone. As of April 2002, 10,154 and 10,625 5′-end ESTs were established from gametophytic and sporophytic stages respectively. These EST sequences were clustered into 4896 non-redundant groups. Database search of the 4896 non-redundant ESTs by BLAST algorithm showed that approximately 40% have similarity to those of registered genes from various organisms, including higher plants, mammals, yeast and cyanobacteria, while the remaining 60% are novel[30].

In conclusion, we think *Porphyra* is one of the best marine organisms for both applied and basic research. However, more research is needed to unravel several other aspects of the biology of *Porphyra*.

1. Sahoo, Dinabandhu, *Farming the Ocean-Seaweeds Cultivation and Utilization*, Aravali Books International, New Delhi, 2000, p. 44.
2. Hanisak, M. D., *World Aquacult.*, 1998, **29**, 18–21.
3. Yarish, C., Chopin, T., Wilkes, R., Mathieson, Fei, X. G. and Lu, S., *Bull. Aquacult. Assoc. Can.*, 1999, **1**, 11–17.
4. Noda, H, *J. Appl. Phycol.*, 1993, **5**, 255–258.
5. Tsujii, K., Kchikawa, T., Matusuura, Y. and Kawamura, M., *Sulphur Amino Acids*, 1983, **6**, 239–248.
6. Mumford, T. F. and Miura, A., in *Algae and Human Affairs* (eds Lemby, C. A. and Walland, J. R.), Cambridge Univ. Press, Cambridge, 1988, pp. 87–117.
7. Levine, I. A., *Bull. Aquacult. Assoc. Can.*, 1999, **1**, 31–33.
8. Yoshida, T., Notoya, M., Kikuchi, N. and Miyata, M., *Nat. Hist. Res. (Spec. Issue)*, 1997, **3**, 5–18.
9. Sahoo, Dinabandhu, Nivedita and Debasish, *Seaweeds of Indian Coast*, APH Publishing Corporation, New Delhi, 2001, p. 283
10. Tang, X. R. and Fei, X. G., *Ocean Limnol. Sin.*, 1999, **30**, 180–185 (In Chinese with English abstract).
11. Mitman, G. G. and van der Meer, J. P., *J. Phycol.*, 1994, **30**, 147–159.
12. Drew, K. M., *Nature*, 1949, **164**, 748–751.
13. Nelson, W. A., Brodie, J. and Guiry, M. D., *J. Appl. Phycol.*, 1999, **11**, 407–410.
14. Nelson, W. A. and Knight, G. A., *Bot. Mar.*, 1995, **38**, 17–20.
15. Nam-Gil, K., *Hydrobiologia*, 1999, **398/399**, 127–135.
16. Sidirelli-Wolf, M., *Bot. Mar.*, 1992, **35**, 251–257.
17. Katz, S., Kizner, Z., Dubinsky, Z. and Friedlander, M., *J. Appl. Phycol.*, 2000, **12**, 535–542.
18. Orfandis, S., *Bot. Mar.*, 2001, **44**, 533–539.
19. Avila, M., Santelices, B. and McLachlan, J., *Can. J. Bot.*, 1986, **64**, 1867–1872.
20. Oohusa, T., *Bot. Mar.*, 1980, **23**, 1–5.
21. Kraemer, G. P. and Yarish C., *J. Appl. Phycol.*, 1999, **11**, 473–477.
22. Chopin, T. *et al.*, *J. Phycol.*, 2001, **37**, 975–986.
23. Kraemer, G. P., Yarish, C. and Carmona, R., *Abstracts Algae 2002*, p. 48.
24. Tseng, C. K. and Sun, A., *Bot. Mar.*, 1989, **32**, 1–8.
25. Wilkes, R. J., Yarish, C. and Mitman, G. G., *Algae*, 1999, **14**, 219–222.
26. Chen, L. C. M., *Bot. Mar.*, 1986, **26**, 435–439.
27. Chen, L. C. M., McCracken, I. R. and Xie, Z. K., *ibid*, 1995, **38**, 335–338.
28. Fujita, Y. and Saito, M., *Hydrobiologia*, 1990, **204/205**, 161–166.
29. Chen, L. M., *Bot. Mar.*, 1987, **30**, 399–403.
30. Asamizu, E., Nakajima, M., Nakamura, Y., Nikaido, I., Kitade, Y., Saga, N. and Tabata, S., *Abstracts Algae 2002*, p. 26.

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