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White Rot Disease and Epiphytism on *Halymenia durvillei* Bory de Saint-Vincent (Halymeniaceae, Rhodophyta) in Culture

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Among the targeted seaweed species for resource development in the Philippines is the red seaweed *Halymenia durvillei* Bory de Saint-Vincent, which is commonly known as "red sea lettuce". Locally, it is known as *lablabig, gayong-gayong, gargarnatis, gamet, aragantiilek,* and *guraman* (Trono and Ganzon-Fortes 1988). Aside from being used as food in many countries (Lewmanomont and Kawaguchi 2002), including the Philippines, *H. durvillei* is also a source of the red pigment *r*-phycoerythrin, as well as a lambda-carrageenan-like polysaccharide (Fenoradosoa et al. 2009). Phycoerythrin is an important food and cosmetic colorant, a therapeutic agent owing to its immunomodulating and anti-cancer activity, and a fluorescent agent, among others (Bermejo Román et al. 2002; Spolaore 2006). The current market value for 1 mg of *r*-phycoerythrin from *Porphyra tenera* "Nori" is US$ 1,030 (SG$ 1,400; Sigma-Aldrich Online Catalog, 2016a) *Lambda*-carrageenan is a type of carrageenan (an integral part of red algal cell wall), but it only occurs naturally to a few species. As with other types of carrageenan, *lambda*-carrageenan is widely used in the food industry as an emulsifier (Fenoradosoa et al. 2009), but it can also act as an allergy suppressant against certain food products (Tsuji et al. 2003). Currently, 5 g of *lambda*-carrageenan is priced at US$ 114.79 (SG$ 156; Sigma-Aldrich Online Catalog, 2016b).

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In view of the high market value of *H. durvillei*, Dr. Gavino C. Trono Jr. of the Marine Science Institute, University of the Philippines (UPMSI), in collaboration with the then Philippine Council for Aquatic and Marine Research and Development (PCAMRD) of the Department of Science and Technology (DOST), developed a culture technology for the sustainable mass production of *H. durvillei*. A booklet entitled '*A Primer on the Land-based Culture of Halymenia durvillaei Bory de Saint Vincent (Rhodophyta)*' (Trono 2010) was published as a culmination of the research and development process. However, in a recent work to refine the technology on vegetative culture as well as in developing the spore to sea-out-planting mariculture of *H. durvillei*, we encountered two challenges that were detrimental to the alga's growth and health: white rot disease and epiphytism. Diseases and epiphytes are perennial challenges to the culture of seaweeds (e.g., *Eucheuma denticulatum* and *Kappaphycus alvarezii*), which often lead to significant losses in biomass and profit for the farmer. We describe herein the nature and progression of the disease, as well as the identity of the epiphytic organism. We also offer possible explanation for the infestation of epiphytes on *H. durvillei*.

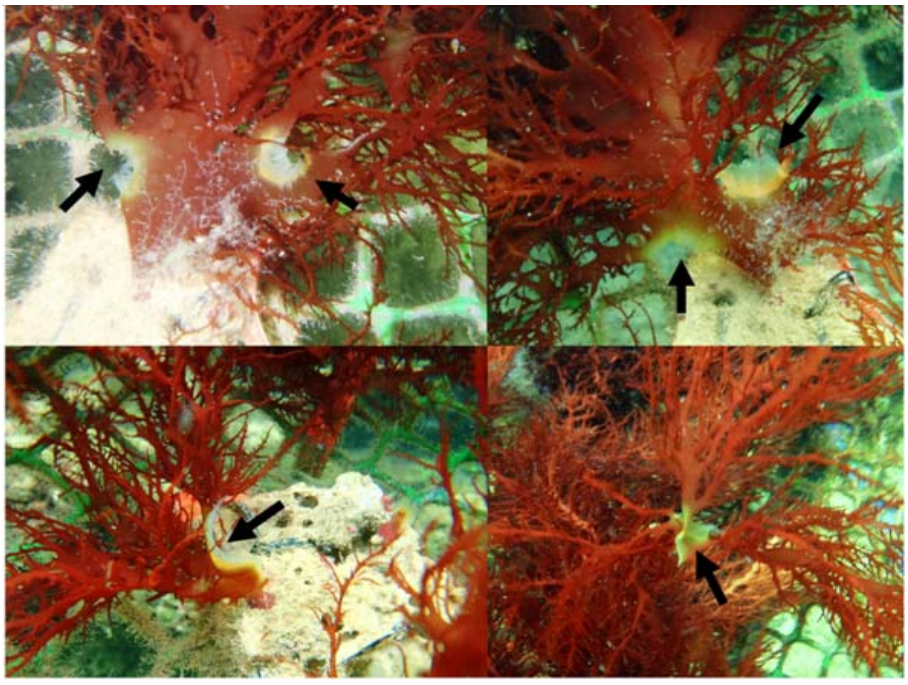

Figure 1. White rot disease infected sea-out-planted carpospores-cultured *Halymenia durvillei* showing discolored and decaying portions of each thallus (arrows). Note that the circular infection often affects the basal portion of the thallus.

We described the occurrence and nature of the disease based on the observations we made on the open sea-out-planted carpospores-cultured *H. durvillei* thalli grown at the Bolinao Marine Laboratory (BML) of UPMSI in Guiguiwanen, Bolinao, Pangasinan. For the land-based (tank) culture of *H. durvillei* using vegetative propagules, we used the methods described by Trono (2008), while the methods utilized on the spore culture to sea-out-planting portion of the project are detailed in Trono (2014). We determined the identity of the epiphyte based solely on the morphological characters of ethanol-preserved samples. Epiphytes were stained with aniline blue and mounted on corn syrup with phenol and were observed under the light microscope.

White Rot Disease on Open Sea-outplanted Carpospores-cultured *H. durvillei*

The white rot disease appears as a semi-circular spot of discoloration on the surface of the thallus. The initial site of infection enlarges while becoming progressively lighter, and its color turns from bright orange to light yellow until it becomes translucent as pigments appear to disintegrate. The affected part of the thallus becomes increasingly soft, with the white to translucent area being the most delicate and often decomposing (Figure 1, arrows). Large and soft infected portions of the thallus are the parts which facilitate the breakage and the consequent loss of the distal portion of the thallus. We noted that, in most cases, the infected parts are found near the base of the thallus. Thus, during rough seas or in the presence of strong waves or currents, the majority of the thalli is broken and washed away, considerably reducing the amount of biomass.

The characteristic symptoms of the disease in *H. durvillei* were similar to the white rot disease reported in *Porphyra*, where *Vibrio* (as *Beneckea*) was indicated as among the possible pathogens (Tsukidate 1977). The discoloration, decay of affected tissues, and consequent loss of biomass in *H. durvillei* due to breakage were also reminiscent of the *ice-ice* disease common among the carrageenan-producing eucheumatoids, *Kappaphycus* and *Eucheuma* (Trono 1974). *Ice-ice* disease in eucheumatoids, in addition to unfavorable environmental conditions, is also associated with pathogenic bacteria (Uyenco et al. 1981), particularly *Vibrio* sp. P11 and *Cytophaga* sp. P25 (Largo et al. 1995; Largo 2002). We were not able to conduct further studies on what environmental factors induced the advent of the disease, but we hypothesize that, as in the case of other seaweed diseases, pathogenic microorganisms may also be involved in the white rot disease in *H. durvillei*.

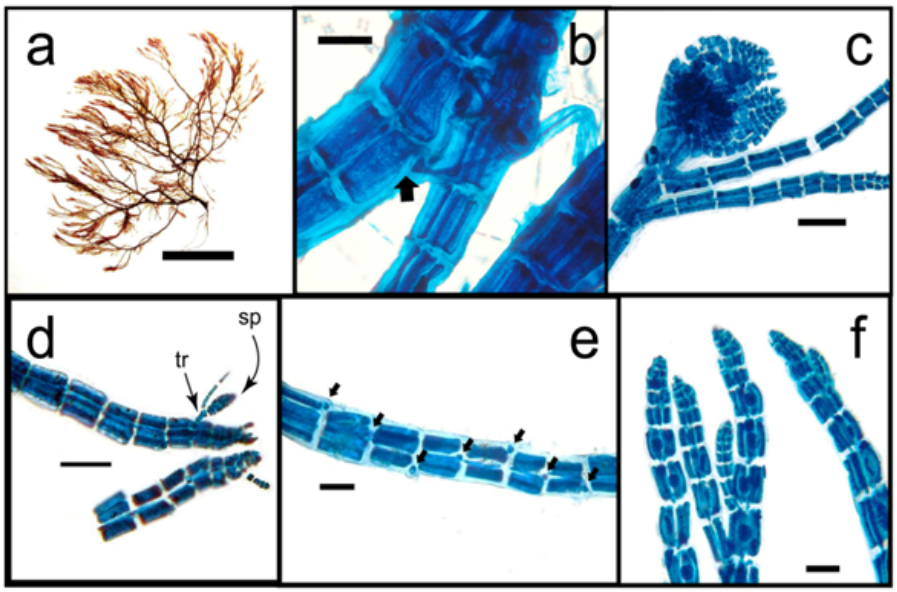

Figure 2. Details of the alga, *Neosiphonia apiculata*, found epiphytic on tank-cultured *Halymenia durvillei*. (A) Dried herbarium specimen; (B) Prostrate portion of the main axis showing rhizoids cut off from the pericentral cells (arrow); (C) Female plant showing ovoid mature cystocarp; (D) Male plant bearing immature spermatangial branch (sp) issuing from trichoblast (tr); (E) Portion of the branch showing spirally arranged scar cells in every segment (arrows); (F) Distal branch portion showing spiral arrangement of tetraspores and the abrupt apical apices (a, scale bar = 1cm; b,c, scale bar = 50µm; d-f, scale bar = 100µm).

Identity of the Epiphyte and Potential Triggers of Epiphytism on Tank-cultured Vegetative *H. durvillei*

The thalli of the epiphytes are brownish-red, mostly erect but sometimes prostrate, and generally less than 3 cm (Figure 2a). The epiphyte's main axes are attached to *H. durvillei* by rhizoids that are cut-off from pericentral cells (Figure 2b). Branching is pseudodichotomous, with each branch abruptly tapering at the apices (Figure 2f). Branch segment has four ecorticate pericentral cells. Trichoblasts are few, rudimentary, and limited at the apices. These are quickly shed, leaving spirally arranged scar cells (Figure 2e, arrows). The species is dioecious. Female gametophytes produce ovoid mature cystocarps (Figure 2c), while male gametophytes produce spermatia on conical spermatangial branches (immature) that develop as a trichoblast fork (Figure 2d). Tetrasporophytes produce

tetrasporangia in slightly spiral series, associated with lateral cover cells (Figure 2f). Despite being relatively larger, the characters of the epiphyte agree with the original descriptions of the polysiphonous alga described by Hollenberg (1968), currently known as *Neosiphonia apiculata* (Hollenberg) Masuda *et* Kogame (Ceramiales, Rhodophyceae) (Tani et al. 2003). The polysiphonous alga, *N. apiculata* appears to be a common epiphyte affecting cultured seaweeds (e.g., eucheumatoids in Calatagan, Batangas and Green Island, Palawan (WJE Santiañez, personal observation)) but may pose as a threat to seaweed farmers, such as the case of the heavy epiphytism in Calaguas Is., Camarines Norte (Hurtado et al. 2006; Vairappan et al. 2008). Heavy epiphytism by *N. apiculata* has negatively affected the development of *H. durvillei,* resulting in stunted growth, forcing us to prematurely discontinue our ongoing study.

Moreover, epiphytism became apparent in March 2013. The occurrence of heavy growth of epiphytes on cultured *H. durvillei* was preceded by algal blooms in the area that are often associated with high nutrient fluxes. During the said period, temperature (29-30°C) and salinity (34 ppt) levels in culture tanks were within optimum levels, but high water nutrient concentration was observed (ammonia: 2.17-3.87 µM; nitrate: 0.89-6.69 µM; phosphate: 2.13-2.54 µM). We believe that high nutrient concentrations coupled with warm water temperature and high incident light during the summer months encouraged the growth and proliferation of *N. apiculata*. In eucheumoid culture in the Philippines, incidence of epiphytism by the alga was also high during summer (Santiañez and Trono, unpublished data).

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Gavino C. Trono Jr. is a National Scientist at the National Academy of Science and Technology (NAST)-Philippines and Professor Emeritus of Marine Science at UP-MSI. Considered as the Father of Modern Philippine Phycology, Dr. Trono has more than 40 years of contribution to the studies on the diversity (taxonomy), ecology, and mariculture of seaweed resources of the Philippines.