B5_001_PF: GROWTH AND SURVIVAL OF THE EPIPHYTIC CYANOLICHEN *Coccocarpia palmicola* IN A TROPICAL RAIN FOREST IN THAILAND

Pitakchai Fuangkeaw,[1,*] Bungon Wannalux,[2] Chaiwat Boonpeng,[1] Kawinnat Buaruang,[1] Kansri Boonpragob[1]
[1]Lichen Research Unit, Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, THAILAND
[2]A Somdet Phra Pinklao Arboretum, Herbology Office, Chatuchak, Bangkok 10900, THAILAND
*e-mail: pfuangkeaw@gmail.com

Abstract: Growth and survival of lichens are essential information for sustainable utilization of lichen resource. Therefore, the main goals of this study were to observe growth and survival of the epiphytic cyanolichen *Coccocarpia palmicola*, and to develop a growth curve of the lichen. A total of 41 thalli having initial diameters between 0.36 and 9.28 cm were observed throughout the period of 11.3 years (October 2004 to December 2015). Almost all thalli grew continuously until reaching the maximum diameters, thereafter many of them degenerated, and some died. Growth rates of the complete thalli at their maximum diameters (GR_{cmp}) averaged 3.34±2.53 mm/y, ranging from 0.09-14.64 mm/y, whereas the degenerated thalli survived to the end of the study period had growth rates (GR_{est}) averaged 1.37±2.52 mm/y, ranging from -3.83-5.87 mm/y. The largest thallus diameter class (>4 cm) showed significantly higher growth rate than those smaller size classes. Larger thalli did not only had higher rates of growth but also live longer than the smaller ones. Consistently, the growth model revealed that the small thalli grow very slowly until the diameters reach 2 cm which lasts about 150 months. Thereafter, the exponential growth starts and reaches the steady state in approximately 600 months (50 years), of which the thallus diameter expands to 15 cm. This model is necessary for estimating thallus age in natural habitat. Most importantly, this study provided fundamental and significant information for chosen lichen thalli, during the exponential growth, for sustainable utilization.

Introduction: Cyanolichens are obligate symbiotic organisms between fungi and cyanobacteria (or blue-green algae). Approximately 10% of lichens worldwide associates with cyanobacteria, which fixes atmospheric nitrogen and supplies to ecosystems. It provides nitrogen source for other organisms especially plants[1, 2, 3], particularly in the tropic where soil nitrogen is limited. Some of them synthesize secondary metabolites that are pharmaceutically active and can be used as antibiotics.[4] However, lichens grow very slowly, ranging from 0.006 mm/y (*Rhizocarpon superficiale*) to 64 mm/y (*Peltigera canina*)[5, 6]. Thus, mass harvesting from natural habitat to meet industrial demand could threaten the lichen to extinction. Therefore, information on growth and survival of lichens is important to utilize lichens sustainably. Although almost all studies in this aspect have been performed in temperate and cold regions[7, 8], nevertheless, it is very scarce in the tropic[9] and become a major constraint on bioresource management.

Thailand presently reports 1,292 species of lichens in the country, however, less than 100 species are cyanolichens.[10] Most of them are found in humid habitats including the tropical rain forest at Khao Yai National Park, where *Coccocarpia palmicola,* is predominant. The objectives of this study were: i) to observe growth and survival of the epiphytic cyanolichen *C. palmicola* in the tropical rain forest at Khao Yai National Park, and ii) to develop a growth model for preliminarily estimate ages of thalli of the lichen in natural habitats.

Methodology: A total of 41 thalli of the cyanolichen *C. palmicola* (Fig. 1a) growing on various trees in a tropical rain forest (Fig. 1b) at Khao Yai National Park in Thailand were observed for 11.3 years (136 months) during October 2004 to December 2015.

Figure 1. a) Thallus of the cyanolichen *Coccocarpia palmicola,* and b) habitat of this lichen in the tropical rain forest at Khao Yai National Park in Thailand (14◦ 05′ N, 101◦ 05′ E).

The lichen thalli had initial thallus diameters ranged from 0.36 to 9.28 cm. They were categorized into 4 diameter classes: 0-1 cm (7 thalli), >1-2 cm (12 thalli), >2-4 cm (14 thalli) and >4 cm (8 thalli). Growth rate of a thallus was calculated by changed in thallus diameter against time. Growth rate was considered in two categories:

Completed growth rate (GR_{cmp}) = $(D_2-D_1)/T$, where D_1 was an initial thallus diameter, D_2 was the largest thallus diameter during the study period and T was the time between D_1 and D_2. This parameter indicated natural growth potential of a thallus until it reaches the maximum diameter before senescence, fragmented, and regenerated or die.

Existing growth rate (GR_{est}) = $(D_2-D_1)/T$, where D_1 was an initial thallus diameter, D_2 was a final thallus diameter that existed, and T was the time between D_1 and D_2. This parameter calculated growth rate of the thallus that remained at the end of the study period, excluding the disintegrated portion.

Growth curve of the lichen *C. palmicola* was constructed based on the method proposed by Zotz[11] for estimating growth curve of the orchid *Dimerandra emarginata*. First, diameters of all thalli after 17 months of observation were plotted against initial thallus diameters. (Fig. 3a)

Second, applied the modified hyperbola I for curve fitting, which obtained a non-linear regression ($Y = 1.2279x/(1+0.0149x)$). By replacing the value "x" of the equation with the smallest initial thallus diameter, then the first Y (Y1) or the first estimated diameter size derived. This diameter was the minimum size of the lichen during the first period of 17 months. Accordingly, the next diameter size for the following 17 months (Y2) was obtained by replacing the value "x" of the equation with the Y1. Consequently, the Y3, Y4, Y5....Y*n* (*n* is number of Y at a constant diameter size) were quantified by replacing the Y2, Y3, Y4....Y*n-1* in the equation, respectively. Finally, the estimated diameter sizes (Y1 to Y*n*) were plotted against time, which represented a growth curve of this lichen. It can be employed for estimating ages of any thalli of *C. palmicola* growing in natural habitats by measuring thallus diameter.

The survival proportion of the thalli during a period of time was calculated by the number of the remaining thalli at a specific time.

The statistically difference for GR_{cmp} and survival time in each diameter size was analyzed with nonparametric statistics based on Kruskal-Wallis at $p<0.05$, and the relationship among data was tested by Spearman's Rank-Order correlation because the data had non-normal distribution, using SPSS software (Version 20, IBM Corp, NY, and USA). The non-linear regression and the growth curve were generated by SigmaPlot v.11 (Systat Software Inc., California, USA).

Results and Discussion: During the 11.3 years of the observation, GR_{est} of all 41 thalli of the lichen *C. palmicola* averaged 1.37±2.52 mm/y ranging from -3.83-5.87 mm/y. Thirty-five of them grew continuously until reaching the maximum thallus diameters (ranging from 0.98-11.58 cm), then most of them degenerated and some died. Six thalli did not increase in size, as they degenerated and died during the study period, thus they had negative growth rates. This lichen showed much lower growth rate comparing with some cyanolichens growing in the same area, i.e., *Leptogium cyanescens* (8.46 mm/y) and *L. marginellum* (7.94 mm/y). However, it had higher rate than *Erioderma mollissimum* (0.58 mm/y) (unpublished data). This is probably due to different biological and/or ecological traits.

Small thalli (0-4 cm initial diameters) needed about 39 months (3.3 years) to attain the maximum diameter averaged 2.64 cm, while large thalli (>4-9.28 cm initial diameters) required about 69 months (5.8 years) to reach the maximum diameter averaged 9.64 cm

GR_{cmp} ranged between 0.09 and 14.64 mm/y, and had the averaged value of 3.34±2.53 mm/y. Larger thalli showed significantly higher growth rates than the smaller ones (Fig. 2a). Thalli with diameters larger than 4 cm had the highest rates of 6.36 mm/y, while the smaller thallus classes had lower values of 3.16 (>2-4 cm), 2.29 (>1-2 cm) and 2.02 (0-1cm) mm/y. Overall, the completed growths were positively correlated with thallus diameters (Fig. 2b). Larger thalli probably had the higher capacity of water storage, then longer photosynthetic period, higher carbon gain, and higher growth rate.[12] Moreover, large thalli also had greater photosynthetic areas[12] and their mycobiont hypha could expand 10 times faster than the small thalli.[13]

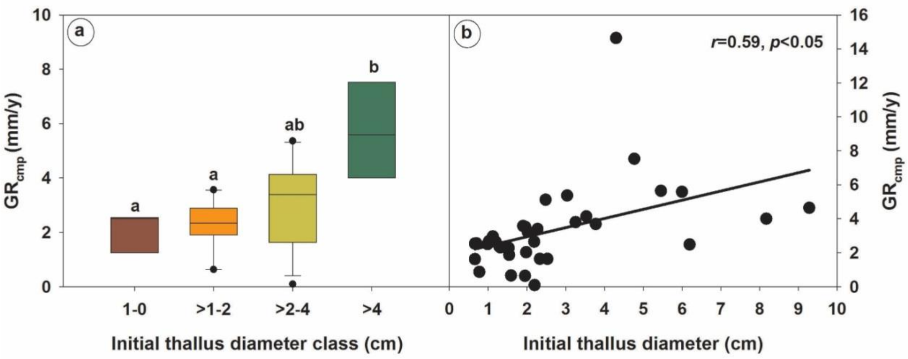

Figure 2. Completed growth rate (GR_{cmp}) of the cyanolichen *Coccocarpia palmicola* in the tropical rain forest during 2004 to 2015, a) GR_{cmp} of each thallus diameter class, and b) the relationship between the GR_{cmp} and the initial thallus diameter. Different letters on the bars indicate a statistically significant difference.

Based on the initial thallus diameters of all thalli and their expansion after 17 months of the observation, the equation: $Y = 1.2279x/(1+0.0149x)$ was generated, which could be used to generate growth curve of this lichen (Fig. 3a, b). This model illustrates that small thalli grow very slowly, they need about 150 months (12.5 years) (Fig. 3b) to expand the thallus up to 2 cm diameter. The exponential growth (Fig. 3b, arrow) starts after 150 months, where thallus diameter is larger than 2 cm. This data is consistent with the measuring GR_{cmp} from the intact thallus, which demonstrated that larger thalli had higher growth rates. The steady state appears at about 600 months (50 years), when thallus diameter is 15 cm. The growth model also reveals that thallus 6 cm in diameter aged at least 274 months (22.8 years). This model is primarily useful for estimating ages and growth phase of *C. palmicola* in natural habitats. Most importantly, for harvesting this lichen at the proper sizes, during the exponential growth phase, for sustainable utilization. However, the model can be improved for higher confidence when more data is available.

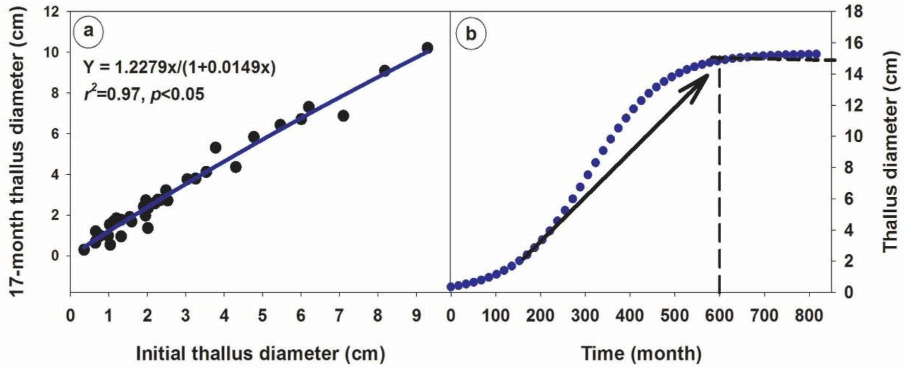

Figure 3. The growth model of the cyanolichen *Coccocarpia palmicola*, a) non-linear regression obtained by applying the modified hyperbola I for fixing curve, and b) growth curve generated by replacing the value "x" with the smallest initial thallus diameter into the equation.

Larger thalli did not only show higher growth rates but also had longer longevity (Fig. 4). They were positively correlated with thallus diameter size (r=0.56, p<0.05). The largest diameter class lived as long as ca. 116±19 months, while the smallest class survived sooner ca. 56±35 months. Number of thalli of each diameter class, 0-1, >1-2, >2-4 cm decreased 71, 71, 42%, respectively, within 6 years of the observation. In contrast, the largest size class survived 100% during the same period of time (Fig. 5), and 4 out of 8 thalli of this class still survived until the end of the study. Overall, only 17% (7 of 41 thalli) survived through the end of 11.3 years observation (Fig. 5). In the tropical moist forest in Panama, the chlorolichen (green-algal lichen) *Parmotrema endosulphureum* also showed similar results.[9]

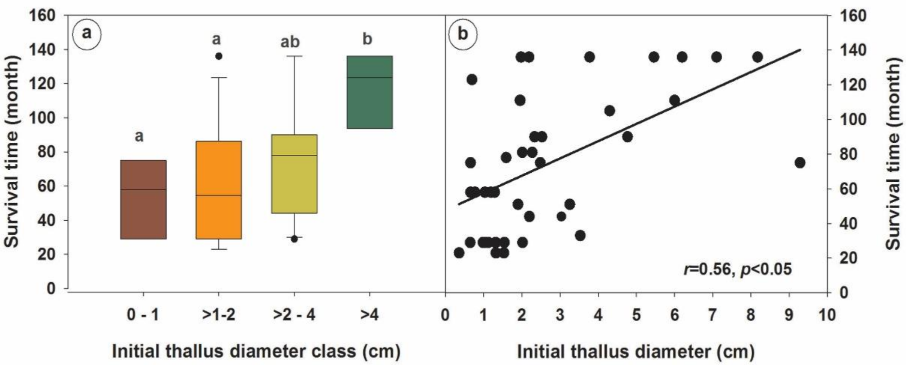

Figure 4. The survival time of the cyanolichen *Coccocarpia palmicola* growing on various trees in the tropical rain forest at Khao Yai National Park in Thailand, a) survival time among four groups of different thallus diameter, and b) the relationship between survival time and thallus sizes. Different letters on the bars indicate a statistically significant difference.

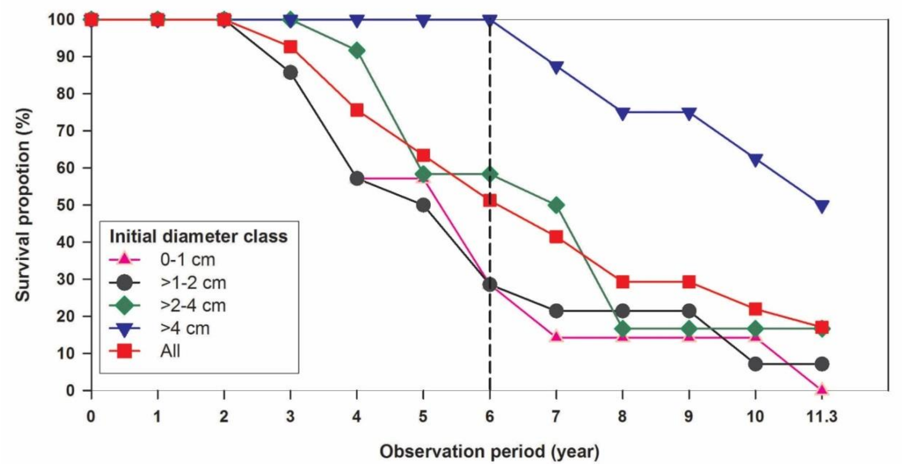

Fig 5. The survival proportion of four diameter-size classes of thallus, and average of all classes of the cyanolichen *Coccocarpia palmicola* during 11.3 years (136 months) in the tropical rain forest.

Conclusion: Only 17% of the thalli of the epiphytic cyanolichen *C. palmicola* survived through the end of 11.3 years of the observation. Most of them degenerated, fragmented or died at different time intervals. Growth rate of all completed maximum thallus size, before disintegrated, averaged 3.34 mm/y. The remaining degenerated thalli at the end of observation had growth averaged 1.37 mm/y. Large thalli did not only had a higher growth rate but also survived longer compared with those small ones. The growth model developed from this study is useful for estimating ages of thalli of *C. palmicola* in natural habitats, and most importantly, for selecting thallus size during the exponential phase, which is the most productive stage appropriated to harvest for sustainable utilization. Nevertheless, additional data in the future, the model can be modified to achieve higher confidence.

References:

1. Nash III TH. Lichen biology. 2008:216-233.
2. Ota´lora MA, Jørgensen PM, Wedin M. Fungal Div. 2014;64:275–293.
3. Rikkinen J. 2015. Biodivers Conserv. 2015;24:973–993.
4. Benedict J.B., Arctic, Antarctic and Alpine Research. 2008;40:15-26
5. Webster M, Brown DH. Lichenologist. 1997;29:91–96
6. Kurma RS, Thajuddin N & Venkateswari C. Afr J Microbiol Res. 2010;4:1408-1411.
7. Sillett SC & Mccune B. Bryologist. 1998;101(1):20-31.
8. Armstrong R & Bradwell T. Symbiosis. 2011; 53:1–16.
9. Zotz, G. & Schleicher, T. Ecotropica. 2003;9:39–44, 2003.
10. Buaruang K, Boonpragob K, Mongkolsuk P, Sangvichien E, Vongshewarat K, Polyiam W, et al. Mycokeys. 2017;23:1–91.
11. Zotz, G. Selbyana. 1995;16:150–154.
12. Larsson P., Solhaug K.A., Gauslaa Y. New Phytol. 2012;194:991–1000.
13. Money N.P. Fungal Biology Review. 2008;22:71–76

Acknowledgments: We would like to thank Mr. Sumrit Senglek, Mr. Mongkol Phaengphech and the members of Lichen Research Unit at Ramkhamhaeng University for their supporting field works. This work was funded by National Research Council of Thailand (NRCT).