DIVERSITY AND ADAPTIVE FEATURES OF CORTICOLOUS LICHENS IN THE HUNDRED ISLANDS, PHILIPPINES

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ABSTRACT

This study is a survey of corticolous lichens in the Hundred Islands, Philippines noting their diversity and morpho-anatomical adaptive features. There were only six islands visited due to accessibility and safety reasons. There are at least thirty-two identified species of lichens; majority is crustose dominated by Graphidaceae lichens followed by pyrenolichens in terms of diversity. Morpho-anatomical features considered to be adaptive include crustose and narrow-lobed foliose growth forms, homiomerous thallus organization, thick upper cortex, and presence of pruina in the cortical or medullary layers.

KEYWORDS: Hundred islands, epiphytic lichens, adaptive features

INTRODUCTION

Lichens are symbioses of heterotrophic fungi (mycobiont) and photosynthetic algae and/or cyanobacteria (photobiont) that must be beneficial to and adaptive for the organisms involved considering that 85% of fungi have resorted to this type of existence (Honegger, 1996). It is known that lichenized fungi have developed complex vegetative structures that enable them to withstand harsh conditions of the environment (Kappen, 1973, 1988). The appearance of lichens is primarily determined by the mycobiont. Lichens generally exhibit three main growth forms: the leafy foliose, the shrubby or hairy fruticose, or the crust-like crustose type.

Believed to have been formed two hundred million years ago, the Hundred Islands is a cluster of islands located in the Lingayen Gulf (16° 12' N, 120° 2' E) in the province of Pangasinan, northern Philippines (Fig. 1). It is composed of 123 islands and islets at low tide (124 at high tide) and covers an area of 1,844 ha. It was declared a national park in January 18, 1940. Over the years, a few species of hardwoods dominated by *Sterculia foetida, S. cordata* and *Vitex parviflora* are able to grow on limestone soil in the islands (Goode, 1912; Peña, 2013). Corticolous lichens, mostly crustose and narrow-lobed foliose forms have inhabited the barks of these trees. Lichens in the Hundred Islands are subjected to excessive radiation and saline water splashes that can bring about desiccation and osmotic stress (Dobson, 2010; Nash, 2008). This study aimed to find out what species of corticolous lichens are present in the Hundred Islands and describe their morpho-anatomical features which enable them to thrive under such harsh conditions.

MATERIALS AND METHODS

Lichen collection. Collection of lichens was conducted in July 2013 at the start of the rainy season of that year. Lichens collected in the islands are expected to have established residence in the tree barks for a number of years. Only six accessible islands were visited: Children's Island, Clave Island, Lopez Island, Governor's Island, Marcos Island, and Quezon Island. This study made use of quadrat sampling technique. The quadrats were subjectively laid for the following reasons: (1) trees were not evenly distributed in some of the islands; hence, we chose only the areas with trees; (2) sampling was done only in safe areas; some areas were off limits due to the presence of snakes. Depending on the size of the island, four to six quadrats with an area each of 25 m^2 were laid. Depending on the abundance and number of species of trees in a quadrat, three to five trees were examined for lichen epiphytes. In quadrats where trees are of the same species, at least three trees regardless of age were surveyed of lichen epiphytes. In quadrats with more than one species of trees, all tree species were observed for the presence of lichen epiphytes. Different tree species vary in their bark characteristics, hence, may vary in lichen epiphytes. Studies have shown that bark structure affects epiphyte colonization and growth (Sheard & Jonescu, 1974; Boudreault et al., 2008). From the base of the tree at a height of 1.0-1.5 m, a clear transparent grid having an area of 600 cm^2 was laid out on the tree trunk where there is abundance of lichens. This method was adapted from Gradstein et al. (2003) although the area of the grid was decreased. Only the lichens that are inside the perimeter of the grid were counted. Lichen samples were then carefully removed from the bark. Tree cuts were covered with colorless nail polish to protect exposed areas from microbial infection. Collections were placed on paper bags, properly labeled and/or tagged.

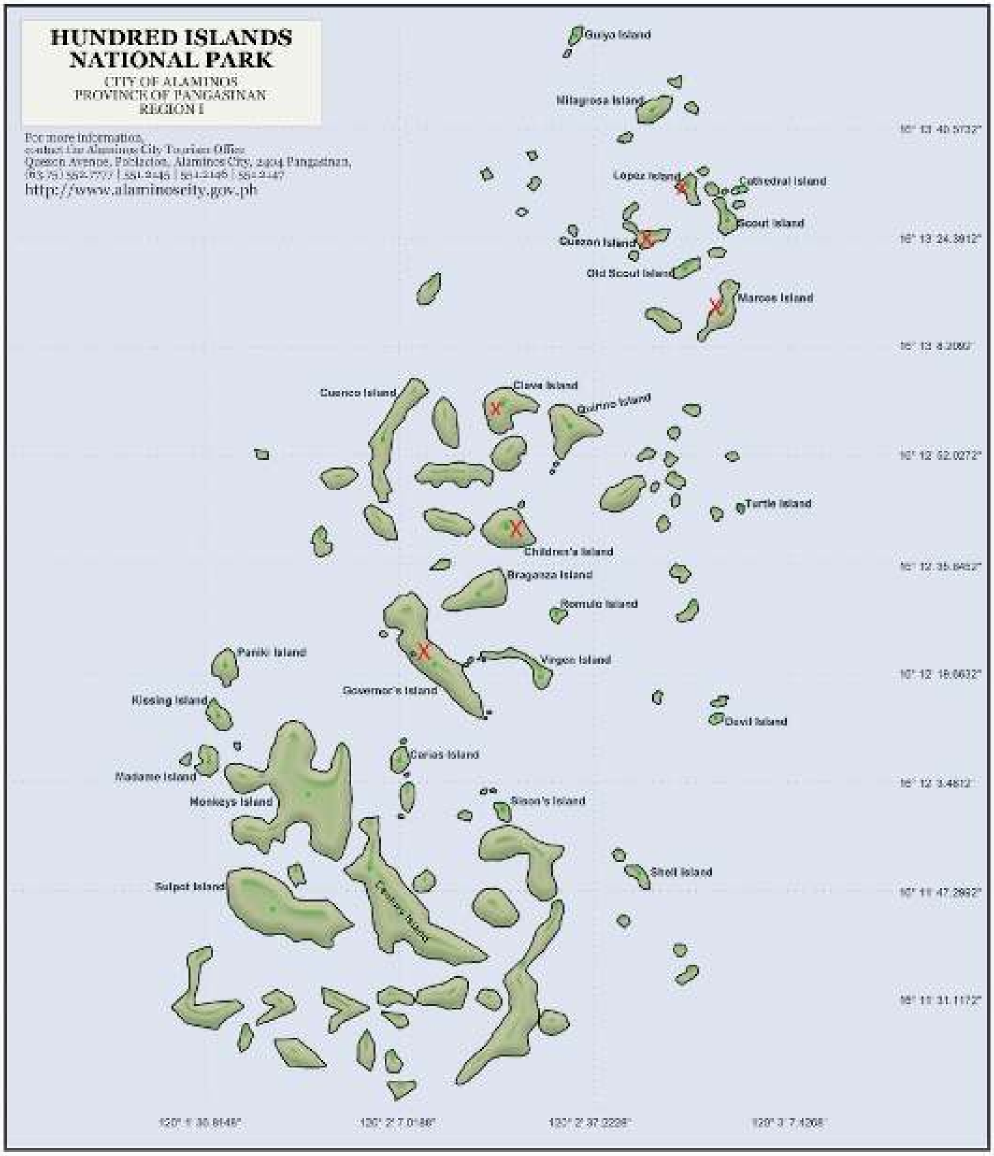

Figure 1. Map of the Hundred Islands. Marked islands (X) are the collection sites (Source: http://www.alaminoscity.gov.ph).

Lichen identification and biodiversity indices. Taxonomic features of the lichens were carefully examined using a Nikon YS100 binocular light microscope and Axio Zeiss Scope.A1. Chemistry of the lichens was determined through spot tests (K, P, C and I) and thin layer chromatography (TLC). Taxonomic keys available online (Sipman, 2005) and in print (Lucking, 2009; Lucking et al., 2009; Aptroot, 2012) were used as guide to identify specimens collected. To verify identification of lichens, reference was made to pictographic guides. For lichens which are difficult to identify, images and features were also sent to consultant lichenologists for the confirmation of initial identification. Taxonomic treatment and characterization of the lichens, however, will be presented in another article. Herbarium vouchers are stored at the lichen herbarium of the Fr. Braeckman Museum of Natural History, Saint

Louis University, Baguio City, Philippines. Biodiversity indices were determined using an online biodiversity calculator (Al Young Biodiversity calculator).

Morpho-anatomical observation. Sectioning of the thallus and ascocarps was done under the Olympus and/or Meiji stereomicroscopes. The Nikon YS100 binocular light microscope and Axio Zeiss Scope.A1 were used to observe the internal anatomy of the thallus. The AxioCam ERC 5s accessory of the Zeiss Scope.A1 was used to take images of the sections. Detailed observation of desired features such as the presence and type of calcium oxalate crystals was done through scanning electron microscopy (SEM) at the Korean Lichen Research Institute (Sunchon National University, South Korea). Similar lichens were also collected from the mainland for comparison purposes.

RESULTS AND DISCUSSION

Diversity of lichens. Identification of specimens collected resulted to at least thirty-two species grouped into two morphological forms, foliose (3) and crustose (29), distributed in 17 genera and belonging to eight families (Table 1). The foliose lichens belonged to family Physciaceae; crustose lichens were distributed in eight families. The family Graphidaceae dominated in terms of diversity with 17 species. The highest number of species was collected from Governor Island; only two species were found in Lopez Island. Consequently, species diversity was highest in Governor Island (1-D=0.8334); lowest in Lopez Island (1-D=0.0384). There is a significant difference in the number of species of lichens present in the islands (df = 5; α=.05; χ =13.476). This result can be partly explained by the Island Biogeography Theory (Wilson and MacArthur, 2001). Among the islands sampled, Governor Island was nearest to the mainland and also the biggest; Lopez Island was the farthest and the smallest island visited. There were more trees (consequently lichens) in terms of species and age that have colonized Governor Island; hence more lichens were found in the island than in the others. Lopez Island had few young trees, with few lichen epiphytes. The Hundred Islands is a protected national park, hence, anthropogenic factors may not be the primary reason for the absence of some lichen species in the islands; this will be attributed more to natural factors that affected the success of trees which can serve as substrates for the lichens to colonize the islands.

Species evenness was highest in Lopez Island (SE = 0.9544); lowest in Children's Island (0. 7798) (Table 1; Figure 1). There are only two species identified from Lopez Island but one is not more dominant than the others as shown by the number of individuals (*Rinodina* =25 and *Fissurina* sp. 1=15).

Table 1. Diversity and distribution of epiphytic lichen species in the Hundred Islands

Growth Form	Taxonomy		Collection sites					
	Family	Species	A	B	C	D	E	F
Foliose	Physciaceae	*Dirinaria applanata*		x	x		x	
		Heterodermia sp.		x	x			
		Pyxine cocoes		x			x	
Crustose	Physciaceae	*Rinodina oleae*	x			x		x
	Coenogoniaceae	*Coenogonium stramineum*			x			x
	Graphidaceae	*Diorygma hieroglyphicum*			x		x	
		Fissurina. dumastii	x					
		F. subcontexta			x			
		Fissurina sp. 1	x		x	x	x	
		Fissurina sp. 2			x			
		Graphis analoga		x				
		G. cincta	x					
		G. consimilis	x					
		G. distincta						x
		G. furcata	x	x	x			
		G. lineola			x			
		Graphis sp, 1			x			
		Graphis sp. 2	x	x				
		Graphis sp. 3	x					
		Cf. Thecaria quassicola						x
		Thelotrema defossum			x			
		Cf. Thelotrema brasiliensis	x		x		x	

	Lecanoraceae	*Lecanora helva*	x	x			x	
	Pyrenulaceae	*Anisomeridium biforme*		x	x			
		Pyrenula sp. 1					x	x
		Pyrenula sp. 2		x				
		Pyrenula sp. 3		x				
		Pyrenula sp. 4	x	x	x		x	
	Ramalinaceae	*Bacidia medialis*	x		x			x
	Roccellaceae	*Opegrapha* sp.						x
	Trypetheliaceae	*Marcelaria cumingii*	x	x	x			x
		Trypethelium aeneum	x	x	x			x
Number of species			14	13	17	2	8	9
Simpson's Index			.3806	0.206	0.1666	1.038	0.5121	0.4882
Dominance Index			0.6194	0.794	0.8334	0.0384	0.4879	0.5118
Shannon-Wiener Index			2.058	2.347	2.597	0.6616	1.663	1.738
Species evenness			0.7798	0.9149	0.9166	0.9544	0.8	0.7857

* A - Children's Island; B - Clave Island; C- Governor Island; D - Lopez Island; E - Marcos Island; F - Quezon Island; x – indicates presence of the lichen

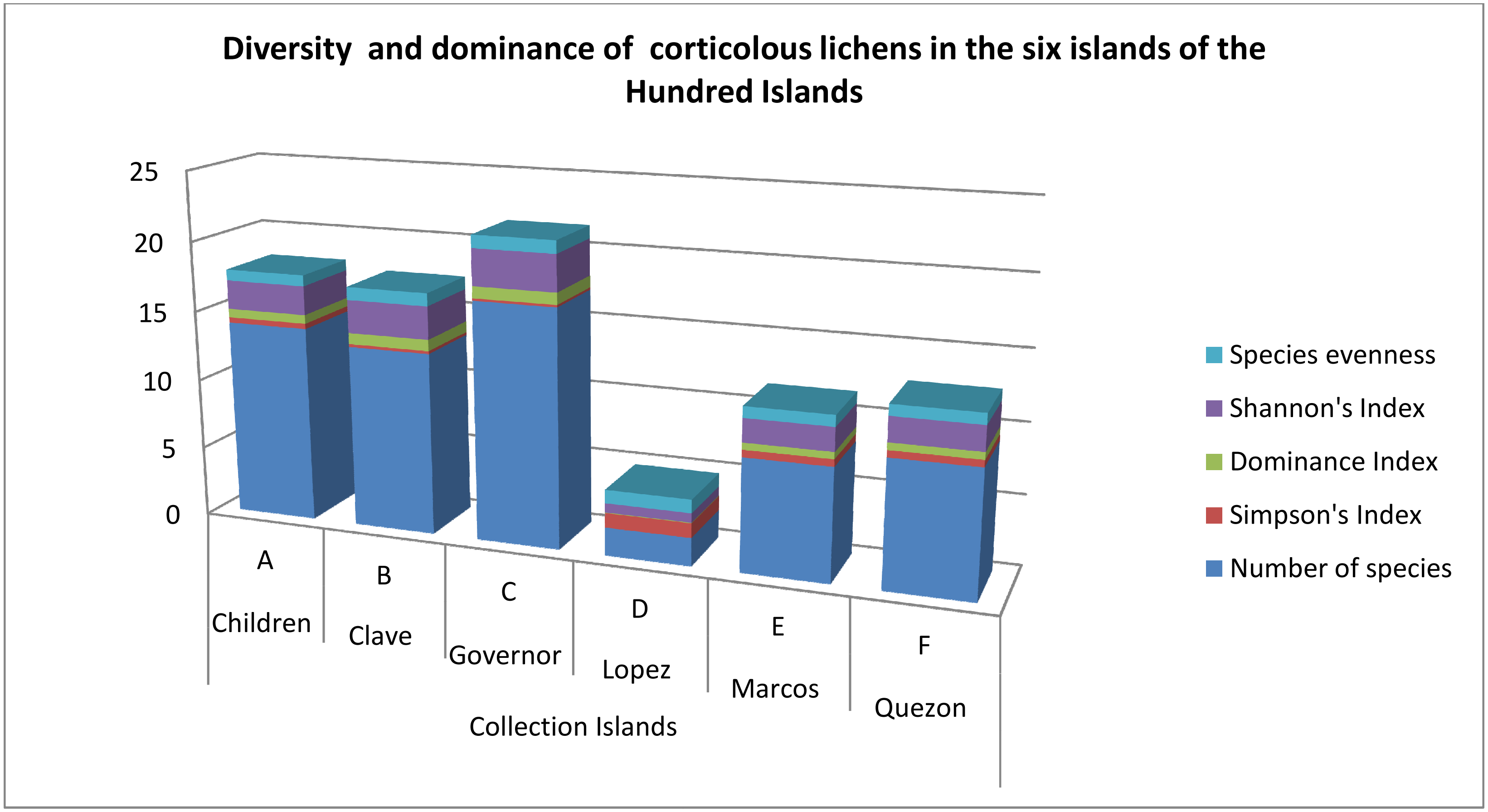

Figure 2. Diversity and dominance of corticolous lichens in the six islands of the Hundred Islands

Table 2. Species similarity between the six islands*

	A	B	C	D	E	F
A		0.179	0.279	0.228	0.377	0.098
B	0.179		0.292	0	0.26	0.017
C	0.279	0.292		0.057	0.164	0.098
D	0.228	0	0.057		0.217	0.038
E	0.377	0.26	0.098	0.217		0.028
F	0.098	0.017	0.098	0.038	0.028	

* A - Children's Island; B - Clave Island; C- Governor Island; D - Lopez Island; E - Marcos Island; F - Quezon Island; values in red shows highest species evenness; those in blue shows the lowest species evenness between two islands.

On the other hand, Children's Island showed 14 species but there was observed dominance by some species (e.g., *Bacidia medialis* = 41; *Graphis* spp. usually have two individuals each). Based on morpho-anatomical and chemical characteristics of the lichens there was not one species that was common among all the islands (Species Similarity = 0) but Graphidaceae and pyrenolichens are well represented in all the islands. Inter-island species similarity was also very low (Table 2). Children's Island had more similar species with Marcos Island than with the others and was least similar with Quezon Island. Clave Island had more similar lichen species with Governor Island but no similar species with Lopez Island. Quezon Island had more similar lichen species with Children and Governor Islands and least with Clave Island. These results certainly are not absolute; they indicated, however, that there is much variation in the species of lichens found in the six islands. Surveys of lichens in the other islands may reveal new and other species. Referring to the map of the Hundred Islands (Fig. 1), distance among the islands seemingly was not a factor that affects species similarity. Again, we suggest that the type and abundance of lichens present in the islands could be affected by other factors such as sunlight exposure, desiccation, and substrate characteristics such as pH, nutrient levels, and possibly even its physical nature (Dobson, 2010).

Adaptive features. Being in a coastal environment, the trees and their epiphytes are subjected to alternating high temperature (during the day) and low temperature (during the night). This implies higher rate of desiccation at day time but lower at night time. Constant splashes of seawater would also provide osmotic stress for the cells of the trees and their epiphytes. Lichens are poikilohydric; their productivity is affected by this nature (Palmqvist et al, 2008). Alternating wet and dry thallus condition causes a see-saw in the metabolic state of symbionts. Prolonging the wet and metabolically active state is essential for their growth and survival. Any mechanism, structural or physiological, that helps them retain the moisture in the thallus is basically essential.

These conditions in the islands prevent a great number of tree species or even epiphytes to grow and survive. The limited number of lichen inhabitants in the six islands of the Hundred Islands confirmed these environmental constraints. Our observation of the lichen structures in comparison to those found in the mainland revealed adaptations of the lichens that help them tolerate the environmental stressors.

Growth form. Majority of the lichens in the islands are crustose (~90%). Crustose lichens are tightly attached to the substratum and part of the thallus is often embedded in the bark. This type of growth form not only makes the lichen thallus partly protected by the bark in which it is embedded but water loss is restricted only to the upper surface (Budel & Scheidegger, 2008). The remaining 10% of island lichens although of the foliose type were narrow-lobed, hence, there was a smaller surface area of the thallus exposed to the environment implying water loss prevention. In the mainland near the coastal areas and approximately 5-8 km away, the same crustose type (graphidoid and pyrenolichens) and narrow-lobed foliose type (*Pyxine* and *Heterodermia*) were observed but no broad-lobed or fruticose types. Generally, therefore, crustose and narrow-lobed foliose type growth forms were favored in hot sunny places like in the Hundred Islands and other low altitude areas because these enabled the lichens to conserve water within the thallus (Figure 3).

Thicker upper cortex. In many lichens, the upper cortex layer varies among lichens. It can be coriaceous (thick and leathery) or membranaceous (thin and papery). In some lichens (e.g., byssoid lichens) this is absent. Many of the island lichens had thicker upper cortex compared to similar lichens in the mainland. This was particularly observed in *Pyxine* collected from the island and from the mainland. The upper cortex of *Pyxine* from Clave Island (Fig. 4A) is definitely thicker than *Pyxine* collected from the mainland (Fig 4B).

In addition, there is a distinct epinecral layer above the upper cortex in these lichens (Fig. 4A). This layer which consists of dead, collapsed photobiont cells make the lichen thallus surface grayish-white due to light reflection (Budel & Scheidegger, 2008). Thicker upper cortex provides better protection against excessive light for the photobiont underneath (Budel, 1990; Kappen, 1988). The presence of an epinecral layer, on the other hand, not only decreases transmission of incident light but the creation of air spaces by the dead cells provides CO_2 diffusion paths under supersaturated conditions (Budel and Lange, 1994).

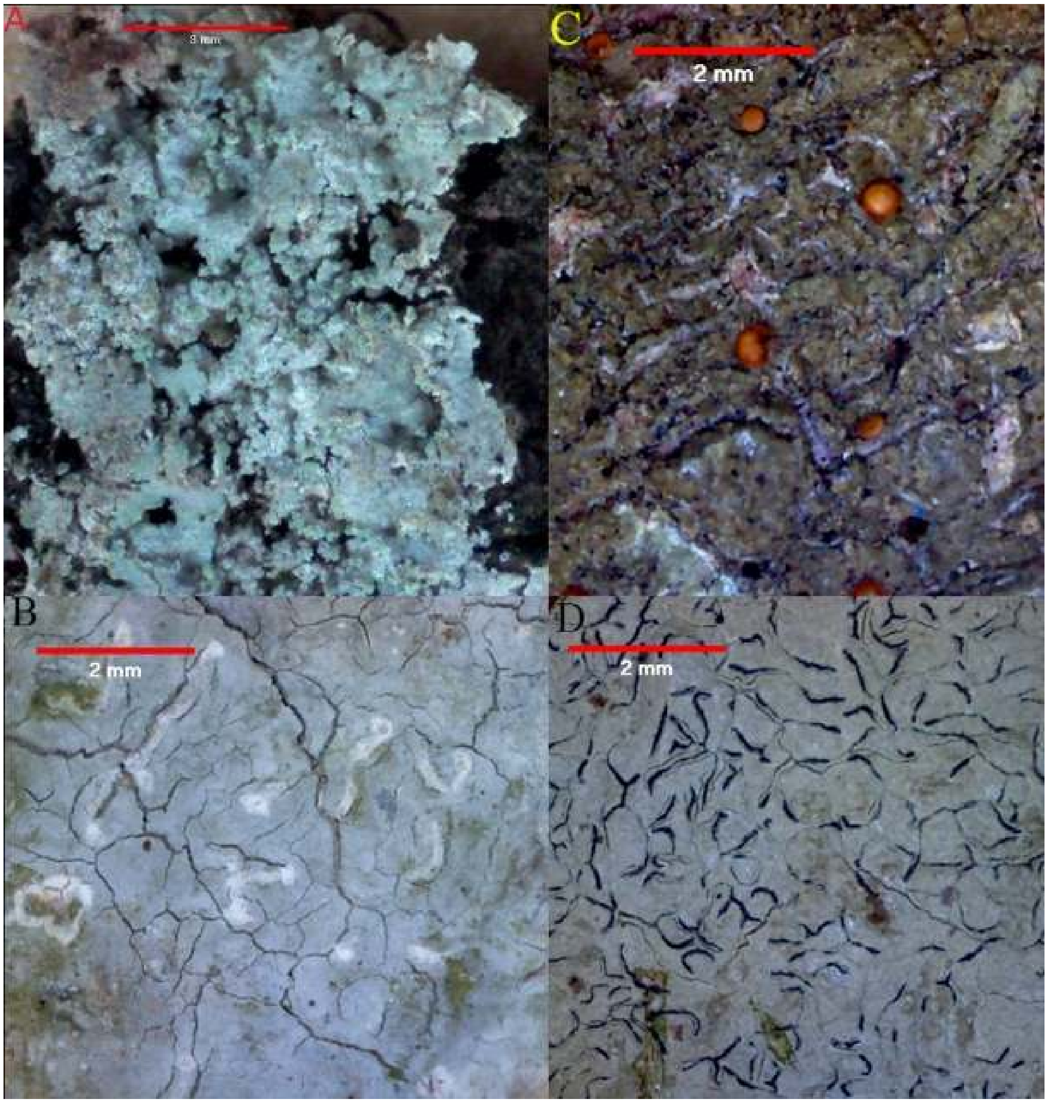

Figure 3. Some lichens collected from the Hundred Islands. A – *Pyxine cocoes* (foliose); B – *Diorygma hieroglyphicum* (Graphidaceae-crustose);C – *Bacidia medialis* (Ramalinaceae -crustose); D – *Graphis furcata* (Graphidaceae - crustose)

Homoiomerous thallus organization. Lichen thallus anatomy may be heteromerous or homiomerous. Heteromerous organization shows four distinct layers: the upper cortex, the algal layer underneath, then the medulla layer followed by the lower cortex. In the homiomerous thallus, however, the algal layer is not distinct; algal cells are scattered between the medullary hyphae extending even towards the lower cortex (Budel & Scheidegger, 2008). This latter type of organization is shown by many crustose lichens and was observed among the crustose lichens collected from the Hundred Islands including those from the mainland (Fig. 5A-5D). However, homoiomerous organization was also observed in the foliose lichens *Heterodermia, Pyxine* and *Dirinaria* collected from the islands. This was not observed in all the foliose lichens collected from the mainland. In homiomerous organization, the photobiont cells occupy deeper layers of the thallus and therefore are accorded more protection by the mycobiont hyphae.

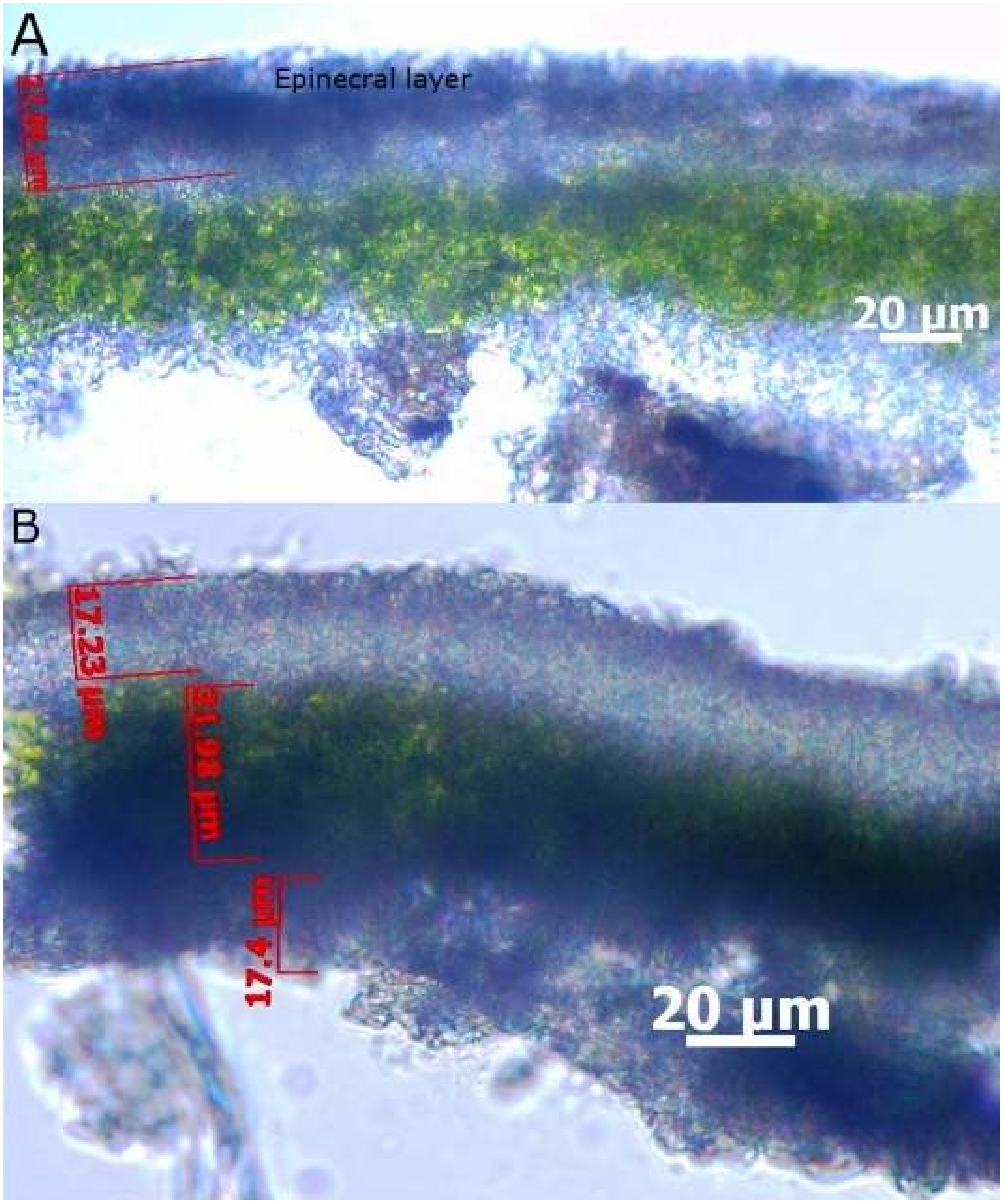

Figure 4. Internal anatomy of *Pyxine cocoes* from Clave Island (A) and from Lingayen City (B). As shown, upper cortex of *Pyxine* from the Hundred Islands is thicker than from the mainland.

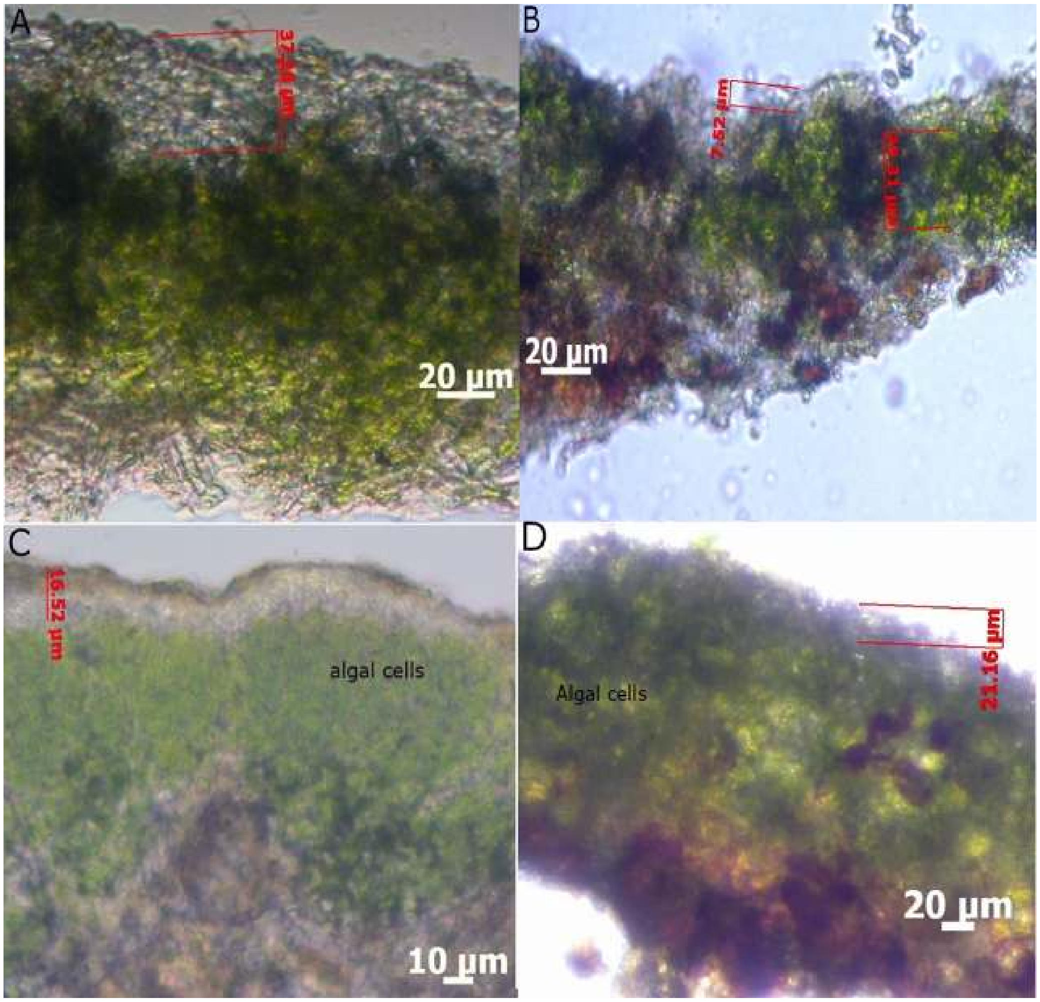

Figure 5. Anatomy of the lichens showing homiomerous organization in three lichens collected from the Hundred Islands (A – *Opegrapha sp*.; C- *Bacidia medialis*, D – *Graphis furcata*) and one from Lingayen City (B – *Rinodina* sp.). As shown, algal cells are distributed throughout the medulla layer in all lichens but this is more pronounced in the lichens from the islands than those from Lingayen City.

Furthermore, this closer proximity between the thalli of the symbiont cells allows faster diffusion of CO_2 and water from the fungal hyphae to the photobiont cells. This definitely is favorable for the photosynthetic process which is vital for the survival of the lichen and in the increase of its biomass (Green et al., 2008; Palmquvist et al., 2008). Both photobiont and mycobiont undergo respiration but the fact that the fungal partner generally constitutes the greater part of the lichen thallus means that it mostly performs the CO_2 releasing metabolic process (Sundberg et al., 1999). Faster rate of CO_2

exchange between the symbionts increases the rate of photosynthesis in the lichen, and consequently its net productivity.

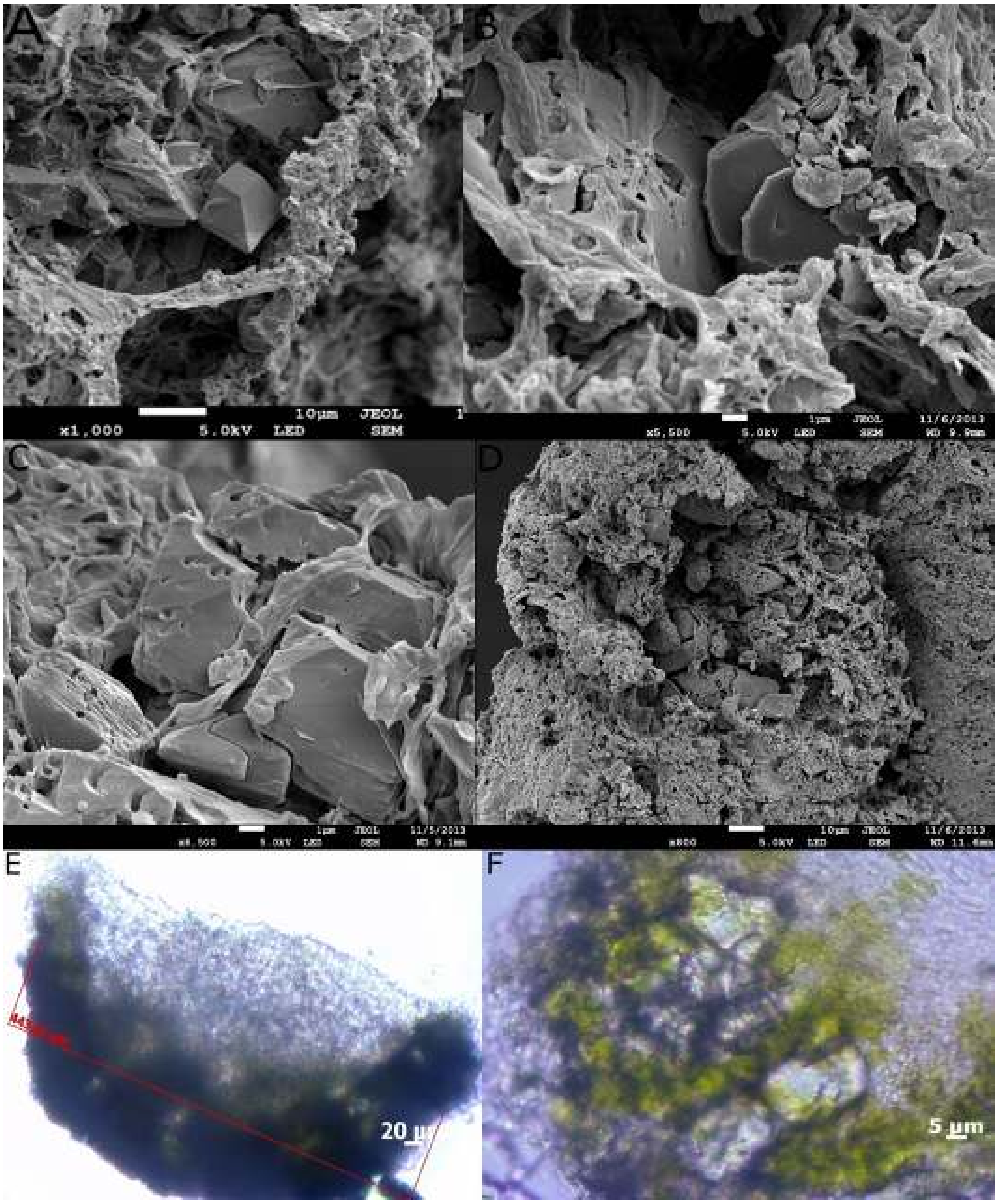

Figure 6. SEM of the calcium oxalate crystals in the lichens: (A) weddelite in *Pyxine cocoes* intertwined with hyphae; (B) whewellite in the thallus of *Thelotrema defossum*; and (C) weddelite crystals on the thallus surface of *Lecanor helva,* and on the apothecium exciple (D). Pruina which appear as crystals are on the disc of an apothecium (E) of *Bacidia medialis* and among photobiont (F) of *Lecanora helva.*

Presence of pruina. Pruina are whitish, powdery materials that are mostly deposited on the surface of the lichens. Under the microscope they appear as crystals. They are mostly composed of calcium oxalate with two crystalline phases: monohydrated whewellite ($CaC_2O_4.H_2O$) and polyhydrated weddellite ($CaC_2O_4(2+x)H_2O$) (Wadsten and Moberg, 1985). We observed two types of calcium oxalate crystals (Figs. 6A-6D). Many are intermittently distributed on the surface of the lichens (Fig. 6C); some are intertwined with the hyphae (Figs. 6A, 6B) or even among the photobiont cells (Figs. 6B, 6F). These calcium crystals are also deposited on the exciple and/or discs of the ascocarps of the lichens (Figs. 6D, 6E).

The production of these pruina in the lichens depends partly on the chemical nature of the substrate such as the calcium content (Budel & Scheidegger, 2008). The islands are coralline; hence, the substrate for the trees is highly calcareous. The calcium content in the tree barks which served as the lichen substrate was not established in this study. Nonetheless, the presence of calcium oxalate crystals is a rule among the island lichens rather than the exception. Compared to lichens collected in the mainland, pruina is more abundant on the surface of the lichen thallus and on reproductive structures of the lichens in the Hundred Islands.

Assuming high calcium concentration in the substrate (in this case, tree bark in the island) of the lichens, this would have affected cytoplasmic calcium concentration of the mycobiont thallus. In addition, saline water splashes may have added to mineral deposits on the lichens. Calcium oxalate formation is a mechanism for the lichen to get rid of the excess calcium (Wadsten and Moberg, 1985; Giordani et al., 2003). The calcium oxalate crystals especially the weddelite crystals are implied as possible source of water in the lichen (Wadsten and Moberg, 1985). Weddelite contains zeolitic water which can leave the crystals when they dry. This water can become available for lichen use especially for the algal cells which are in close proximity to these weddelite calcium oxalate crystals. The size of the crystals and their proximity to the algal cells has been shown as important factors in this mechanism (Clark et al., 2001). The weddelite crystals alternately trap and release zeolitic water. During hot days, the water is released as vapor which can be trapped by the algal cells. Green algal cells, which are predominantly the photobiont in the lichens collected from the islands, have the capacity to use water vapor for photosynthesis (Nash, 2008).

Besides their role in trapping water for the lichen, calcium oxalate crystals also provide mechanical protection, serve as deterrents against herbivores (Reutimann and Scheidegger, 1987) and provide protection against excessive light by deflecting some light rays from reaching the lichens (Purvis, 2000).

CONCLUSIONS

A survey of the corticolous lichens in six islands of the Hundred Islands National Park shows so much diversity that it encourages further study of the lichens in the other islands. There is a need to determine the factors that cause very low species similarity between the islands. The lichens have structures that enable them to withstand the stresses caused by excessive radiation, saline precipitation, and possibly alkaline environment. All these features cooperatively interplay to provide protection against predators, maintain lichen thallus moisture content, and facilitate gas exchanges that enable metabolic processes to occur thereby allowing lichen growth, survival, and ultimately existence.

ACKNOWLEDGMENT

This study was made possible through the University Research Grant (URG) from Saint Louis University and from the Korean Lichen Research Institute, Sunchon National University, Suncheon, South Korea. Pangasinan State University assisted the researchers in securing the permit to collect lichens from the Lingayen local government unit and from the Hundred Islands Park Management.

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