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# Identification and diversity of the fruticose lichen *Usnea* in Kalinga, Luzon Island, Philippines

**Galinato MGM, Mangubat CB, Leonor DS, Cababa GRC, Cipriano BPS, Santiago KAA**

*Department of Biological Sciences, Institute of Arts and Sciences, Far Eastern University, Manila*

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## Abstract

The mountains of Kalinga are home to countless unprecedented organisms. Its cool temperature and high elevation provide the perfect niche for such organisms to survive and these include the lichens. Kalinga harbors a wide variety of lichens stretching from crustose, foliose and fruticose types. Interestingly, the genus *Usnea* is one of the most commonly found fruticose lichens in the northern part of the Philippines. However, these organisms remain neglected and hence limited studies have been document. In fact, not a single species of *Usnea* has been recorded in the province of Kalinga. In this study, 289 *Usnea* samples were collected from four out of eight municipalities of Kalinga. Following published identification keys, 25 species were identified using the conventional morphological characterization and thalline spot test. Furthermore, the diversity of *Usnea* in the province was also determined through the use of biodiversity indices (i.e., Shannon-Weiner index & Pielou's index) accounting for the diversity, evenness and dominance of species. In this study, the municipality of Pasil shelters the most diverse *Usnea* species (H = 2.696), while Balbalan has the highest species evenness (e = 0.920).

**Key words** – distribution – diversity index– fungal diversity – lichen taxonomy

## Introduction

The province of Kalinga has a total land area of 3,119 sq. km., comprising 17% of the Philippine Cordillera Administrative Region (DILG–CAR 1999). The mean temperature of this mountainous province ranges from 17 to 22°C with an altitude of ~2,300 meters above sea level (masl). Kalinga possesses diverse types of terrestrial ecosystems, which is an important factor contributing to the proliferation of various macro– and microorganisms including the lichens.

Lichens are one of the most established symbioses in nature composed of mycobiont (fungus) and a photobiont (algae or cyanobacteria) (Nash 2008). They exhibit many survival strategies that make them widely distributed in many types of environment, sometimes even allowing their growth on extreme ones (Hall & Andre 2001, Lalley & Viles 2005, Garvie et al. 2008). Feuerer & Hawksworth (2007) recognized ~20,000 species of lichens worldwide, wherein ca. 790 (2.53%) are present in the Philippines (DENR 1999). The preliminary compendium on Philippine lichens was present by Wainio in 1909. This then raised the need to cover more places in the country where lichens must be abundant, specifically in the Northern Philippines. However, limited studies have

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Corresponding Author: Mark Gabriel M. Galinato – e-mail – markgabrielgalinato@gmail.com

undertaken in the country since then (Santiago et al. 2010). This resulted to an incredible gap in the series of lichen literature in the country making this most celebrated work out–of–date in many aspects.

The lichen genus *Usnea* has ca. 800 taxa distributed worldwide (Clerc 2004), but only 31 have been reported in the country (Wainio 1909, Herre 1963, Santiago et al. 2010). Due to its complex and heterogenous morphology, *Usnea* has been considered exceptionally difficult in taxonomy" and hence has gained a bad reputation (Clerc 1998). However, the unique functions and applications of *Usnea* in the field of medicine, biomonitoring, dye and perfume making, to name a few, have paved way to the resurgence of this genus. Still and all, taxonomy is the starting point of any basic or applied biological research (de Moraes 1987). Furthermore, the construction of distribution maps has been an important link to the identification of organisms. These maps represent the presence and/or diversity of species in a certain area and thus follow the identification of those species (Franklin 2009). Therefore, this manuscript takes a detailed study of the taxonomy and distribution of the fruticose lichen *Usnea* in the province of Kalinga, Luzon Island, Philippines.

## Materials & Methods

### The study site

Kalinga (17°45'N 121°15'E) is located centrally in the Philippine Cordillera Administrative Region (Fig. 1) composed of 8 municipalities namely, Balbalan, Lubuagan, Pasil, Pinukpuk, Rizal, Tabuk, Tanudan and Tinglayan, and collectively consists of 153 barangays.

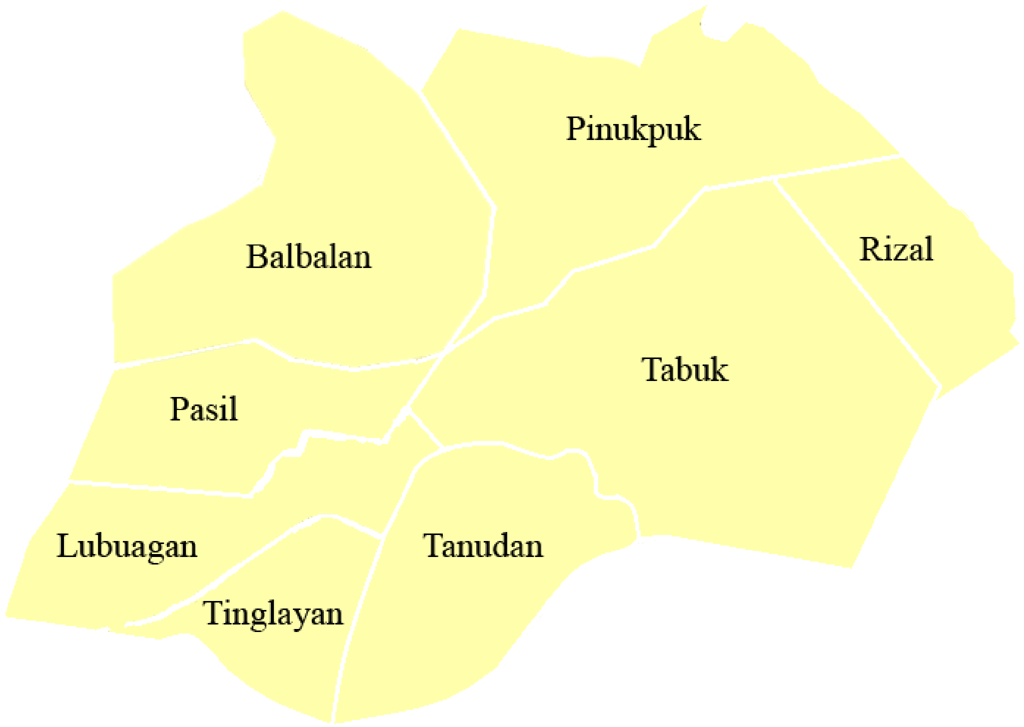


**Fig 1** – Map of Kalinga, Philippines showing the eight municipalities of the province.

### Collection and preparation of *Usnea*

The collection of *Usnea* included the attachment organs or holdfast (Goward et al. 1994). The lichen samples were immediately placed in acid-free paper bags and air–dried afterwards. Extraneous matters were removed prior to identification and the samples were assigned with field numbers for identification (i.e., USN0001–USN0289).

**Characterization and identification of the lichen samples**

Collected samples were initially characterized based on morphological tests following the published identification keys and taxonomic studies by Halonen et al. (1998), Halonen (2000), McCune (2005), Randlane et al. (2009), Ohmura et al. (2010) Ohmura (2012; 2014), Truong et al. (2011), and Shukla et al. (2014). In addition, the thalline spot test (K, KC, and C test) was done to further confirm the identity of species. A razor blade was use to cut open the thallus, exposing the cortex, medulla and/or central axis. Then, chemical reagents such potassium hydroxide (KOH) and/or 5.25% sodium hypochlorite (NaOCl) were directly spotted onto it. For the K test, a drop of KOH applied to the open thallus. For C test, a drop of NaOCl was spotted on another exposed thallus. Finally, for KC test, a drop of KOH followed by a drop of NaOCl was spotted onto another exposed thallus. Any change in color was recorded.

**Biodiversity assessment and distribution maps**

Several standard diversity indices are used in literatures involving biological diversity and ecological monitoring. Correspondingly, Shannon–Weiner Diversity Index (H) was used to assess the species diversity of each municipality of Kalinga (Shannon 1948):

$$H = -\sum_{i=1}^{k} pilogpi$$

Further, Simpson's Index (D) was also used following the first index since it shows weight to common or dominant species (Simpson 1949):

$$D = \frac{1}{\Sigma P_i^2}$$

Finally, Pielou's Evenness Index was used to measure the species evenness (Pielou 1966) following the formula:

e = H / In S

H = Shannon-Wiener diversity index
S = total number of species in the sample

A distribution map was constructed for each species reported in this study using DIVA–GIS, a software for mapping and analyzing spatial data in order to study the distribution of organisms to depict species frequency and to elucidate ecological patterns (Hijmans & Elith 2012).

**Results**

A total of 289 specimens were collected from four out of eight municipalities of Kalinga. These samples were collected from different substrates at different elevations. From these specimens, 25 *Usnea* species were identified (Table 2).

All collected lichen specimens were epiphytic; substrates were recorded either as barks or twigs belonging to the plant genera *Shorea* (Philippine Mahogany), *Areca* (Palm tree), *Pinus* (Pine tree) and trees locally known as "Balasang". Fifteen species out of 106 samples were identified from Lubuagan, 21 species out of 109 samples from Tinglayan, 19 species out of 46 samples from Pasil, and 11 species out of 28 samples from Balbalan (Table 1). No specimens were collected from Pinukpuk, Rizal, Tanudan, and Tabuk.

**Table 1** GPS Data, elevation, substrate, and number of specimens and species collected per municipality.

| Municipality | Coordinates | No. of specimens collected | No. of species identified | Elevation (masl) | Substrate/s |
|---|---|---|---|---|---|
| **Lubuagan** | 17°20'N 121°10'E | 106 | 16 | 636; 898 | M, P |
| **Tinglayan** | 17°19'N 121°9'E | 109 | 21 | 523; 535; 943 | Ba, Bu, M, P |
| **Pasil** | 17°20'N 121°11'E | 46 | 20 | 886 | M, P |
| **Balbalan** | 17°28'N 121°13'E | 28 | 12 | 844 | Bu, M |
| **Rizal** | 17°30'N 121°35'E | 0 | 0 | 170 | None |
| **Pinukpuk** | 17°30'N 121°16'E | 0 | 0 | 448 | None |
| **Tanudan** | 17°18'N 121°14'E | 0 | 0 | 460 | None |
| **Tabuk** | 17°32'N 121°32'E | 0 | 0 | 198 | None |
| **Total # of specimens collected:** | | **289** | | | |

Legend: Balasang (Ba), Bua (Bu), Mahogany (M), Pine (P)

In this study, *Usnea fragilescens* was observed to be the most frequent to occur in the four municipalities while *U. articulata*, *U. cornuta* and *U. diplotypus* were noted to be present in the four sites (Table 2). Likewise, species that are unique in a particular site were also classified. For instance, *U. cavernosa* and *U. lapponica* were exclusively seen in Pasil.

**Table 2** Occurrence of *Usnea* spp. per municipality

| ***Usnea* spp.** | **Lubuagan** | **Tinglayan** | **Pasil** | **Balbalan** |
|---|---|---|---|---|
| *U. articulata* | + | + | + | + |
| *U. baileyi* | + | + | + | + |
| *U. barbata* | + | + | + | + |
| *U. cavernosa* | - | - | + | - |
| *U. ceratina* | - | + | - | - |
| *U. chaetophora* | + | + | + | + |
| *U. cornuta* | + | + | + | + |
| *U. dasaea* | + | + | + | - |
| *U. dasypoga* | - | + | + | - |
| *U. diplotypus* | + | + | + | + |
| *U. esperantiana* | - | + | - | - |
| *U. flammea* | + | + | + | - |
| *U. flavocardia* | + | + | + | + |
| *U. fragilescens* | + | + | + | + |
| *U. glabrata* | + | + | - | + |
| *U. glabrescens* | + | + | - | - |
| *U. hirta* | + | + | + | + |
| *U. lapponica* | - | - | + | - |
| *U. longissima* | + | + | + | - |
| *U. nidulans* | - | - | + | - |
| *U. rubicunda* | + | + | - | + |
| *U. schadenbergiana* | - | - | + | + |
| *U. silesiaca* | - | + | + | - |
| *U. subscabrosa* | + | + | + | - |
| *U. substerilis* | - | + | + | - |
| **Total:** | **16** | **21** | **20** | **12** |

Legend: present (+), absent (-)

Using the three biodiversity indices, a comparison among municipalities in terms of species diversity, dominance and evenness was formulated. Pasil had the highest species diversity and species dominance while Balbalan was shown to have the highest species evenness among the four municipalities (Table 3).

**Table 3** Biodiversity Indices value of the four municipalities in Kalinga

| Municipalities | Shannon-Wiener Index value (H) | Simpson's Index value (D) | Pielou's Index value (e) |
|---|---|---|---|
| **Tinglayan** | 2.535 | 7.720 | 0.820 |
| **Lubuagan** | 2.090 | 4.994 | 0.754 |
| **Pasil** | 2.696 | 10.372 | 0.899 |
| **Balbalan** | 2.286 | 8.430 | 0.920 |

## Discussion

The high elevation of Western Kalingan mountains harbor a highly diverse lichen flora consisting of different communities. It is vastly forested by trees and maintained in a low temperature, making it suitable for fruticose lichens to flourish in. Since they remain underexplored in the country (Santiago et al. 2010), only few studies have been carried out regarding their diversity. Kalinga Province comprises eight municipalities. In this study, total of 289 lichen specimens were collected from four municipalities, whereas the remaining four showed no presence of lichens of the fruticose type (Table 1). Accordingly, the physiology of lichens has been reported by Giordani and Incerti (2008) to strongly be related to macro– and microclimatic factors. For instance, the primary sources for the hydration of lichens are the relative air humidity and occasional rainfall (Giordani & Incerti 2008). This might give an explanation as to why fruticose lichens such as *Usnea* can only be found in places with high elevation, in addition to environments with minimal pollution.

The site with the highest number of collected lichen samples was Tinglayan. The municipality of Tinglayan together with Lubuagan are both mountainous with steep slopes and are both located deep in the Central Cordilleras (DILG–CAR 1999). On the other hand, no *Usnea* was found in Tabuk, Pinukpuk, Tanudan and Rizal due to low elevation (Table 1) and probably, the prevalence of mosses. Species invasion is one of the major threats of lichen diversity (LaGreca & Stutzman 2006) since their slow-growing nature discourages them from spatial competition (Hale 1983, Dent et al. 2013). Meanwhile, although the upper portion of Pinukpuk is suspected to harbor good forest growth where *Usnea* can occur, the aftermath of the landslide observed had made the area inaccessible for biological sampling. Similarly, almost all of the lands in Tanudan have been used for irrigation.

Pasil, wherein 46 specimens were collected representing 20 species, has the highest diversity of *Usnea* among the four municipalities (H = 2.696) (Table 3). In comparison with the other municipalities with higher number of species and individuals, Pasil has the most evenly-distributed species. Hence, the number of species and individuals alone do not actually account for the diversity of a site. Likewise, Pasil, with the most balanced common and rare species, has the highest species dominance while Balbalan, with the Pielou's index value of 0.920, has the highest species evenness (Table 3).

All four municipalities represent the rich biodiversity that the province shelters and both the topography and climate of the areas upkeep the existence of *Usnea*. Kershaw (1985) correlated the roles of environmental conditions (e.g., climate, substrate, light, moisture) to lichen distribution. The climate in Kalinga is classified under Type III characterized by a short dry season and a pronounced wet season (DILG–CAR 1999). Also, the province is predominant with great variety of trees that support organisms that inhabit them as exemplified by the presence of all lichen types found in characteristic forests such as Kalinga.

In general, the ecosystems in these places are perfect niche for *Usnea* but the uncontrollable destruction of habitats has started the threat on this lichen genus. The collected *Usnea* species, being the fastidious organisms that they are, were greatly affected by several anthropogenic activities currently undergoing in some municipalities in the province. These activities are mainly irrigation, road development, mining, and logging. In contrast, landslides and other geographical phenomena were also experienced in Kalinga. Landslides abruptly change the topography of such places, therefore altering its biodiversity (Sassa & Canuti 2009). The effects of the destruction of habitats on the diversity of lichens in the province made the data gathered in this study more prized because perhaps, it would not take long enough until the remaining municipalities be greatly affected by such activities. Additionally, it is worth knowing that the occurrence of *Usnea* in this study was observed starting from around 600 masl. Low–elevated areas in the province, particularly Rizal (170 masl), have been documented for full coverage of Kalinga.

Through morphological and biochemical characterizations, 25 species were identified (Table 2, Table 3). The identification of lichens, especially in the genus *Usnea*, is done precisely and in an orderly manner since many lichen taxa both morphologically and biochemically resemble others. Lichens display wide variety of shapes, sizes, and forms. The appearance of lichens is usually determined by the organization of filaments of the fungus (Nash 2008). In the case of *Usnea*, Nash (2008) described that its thalli, which contains the evenly distributed mycobionts and photobionts, make up a branching pattern that strand out from the surface of its substrate. *Usnea*, having been considered as one of the fruticose lichens to possess peculiar anatomy, has a strong central strand of arranged hyphae that provide mechanical strength along the longitudinal axis (Nash 2008). Moreover, the thallus of *Usnea* consists of several layers. Outstandingly, the central axis is a characterizing feature of the genus although not evident in all species particularly in *U. baileyi* in this study (Fig. 2).

**Fig 2** – Distinguishing features of *Usnea baileyi* highlighting its fistulose axis (←).

Interestingly, *U. fragilescens* occurred in most sites (Table 2) (Fig. 3). This species was found in both lower and higher elevations, making it the dominant species in the study that is capable of growing along the shared parameters of the genus. It has also been reported in other studies to thrive in low elevations, although lower than 500 masl, and in cloud forests at 2,805 masl (Herrera–Campos et al. 2001).

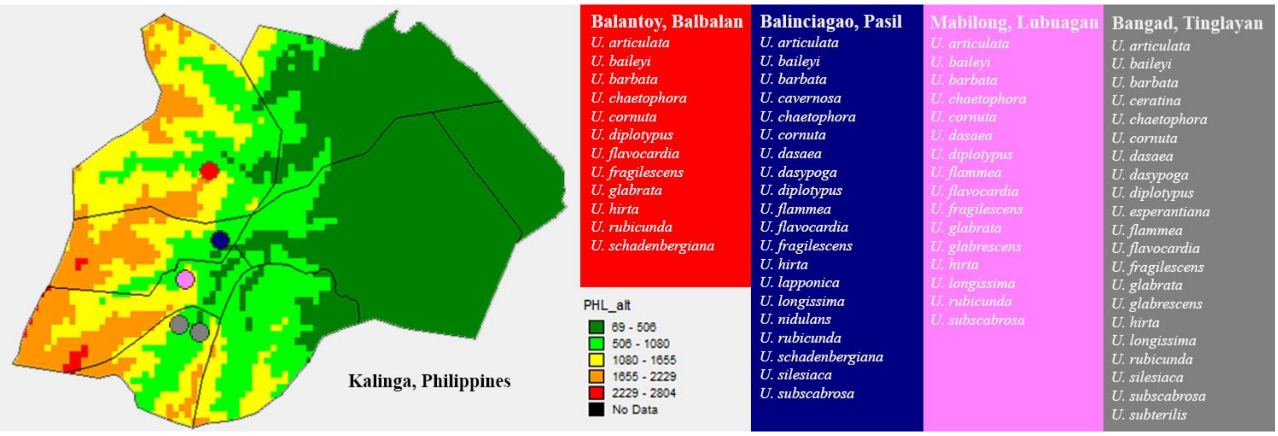


**Fig 3** – Distribution of *Usnea* spp. in Kalinga, Philippines

In this study, some species such as *U. glabrescens*, *U. schadenbergiana*, and *U. substerilis* have been recorded to occur in only two municipalities. In addition, as previously mentioned, only one specimen for *U. cavernosa* and *U. lapponica*, has been collected. Fewer specimens were collected in this study and were identified as *U. schadenbergiana*, *U. glabrescens*, *U. substerilis*, *U. silesiaca*, *U. cavernosa*, and *U. lapponica*. Buckley (2011) and Dent et al. (2013) pointed out that the presence of epiphytic lichens on their habitat depends on their dispersal abilities. This, perhaps, is the reason for the low number of collected specimens. Their dispersal abilities might have been poor and so the distance between the populations of *Usnea* might have potentially limited their rate of migration (Johansson & Ehrlen 2003). In addition, the ability to disperse spores to a tree does not assure the successful establishment of lichens as stated by Dent et al. (2013).

It is also important to emphasize that some species which are considered to be synonymous to another (i.e., *U. pensylvanica* to *U. rubicunda*) are sometimes proven to be monophyletic through molecular studies, i.e., morphologically similar but different at the molecular level (Ohmura 2008). Other species that are morphologically and biochemically similar to one another (Randlane et al. 2009), e.g., *U. diplotypus*, *U. lapponica*, and *U. substerilis*, clearly elucidate the genus' taxonomic challenge (as being regarded as "exceptionally difficult"). Thus, this makes the identification of medullary compounds of no use in distinguishing these species (Randlane et al. 2009). In this study, the identities of the collected *Usnea* samples were carefully determined. Almost all the samples were easily identified using the conventional identification keys. Other specimens (data not shown), which could not be identified up to the species level, must then be subjected to molecular identification to achieve full certainty of their identity.

As of today, *Usnea* remains problematic in terms of taxonomy. This study had given new information on *Usnea* and biodiversity in a wider sense. In fact, this is the first study made on the Philippine *Usnea* in Kalinga. Soon enough, several studies could rise in accordance to this and, perhaps, shed more light to the complex nature of *Usnea*.

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