Lichen specimens: collection and preservation

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Abstract: Collection of lichens in a field, making specimens and preservation them in the herbarium are introduced according to the methods applied in the National Museum of Nature and Science (TNS).

Keywords: growth form, herbarium, substrate

Introduction

Lichen is a symbiotic organism composed of fungus and alga (and/or cyanobacterium). It distributes from tropics to polar regions and covers ca. 6% of whole land on the earth (Haas & Purvis 2006). The substrates where lichens growing are soil, rock, concrete, tree bark, leaf, and so on. The growth forms of lichens can be roughly divided into three: crustose, fruticose and foliose (Fig. 1). The size of thallus ranges from a few mm in diameter (e.g. *Phylliscum japonicum*) to over 3 m in length (e.g. *Usnea longissima*). Lichen specimens should be made taking consideration of such features and placed in the herbarium with appropriate size.

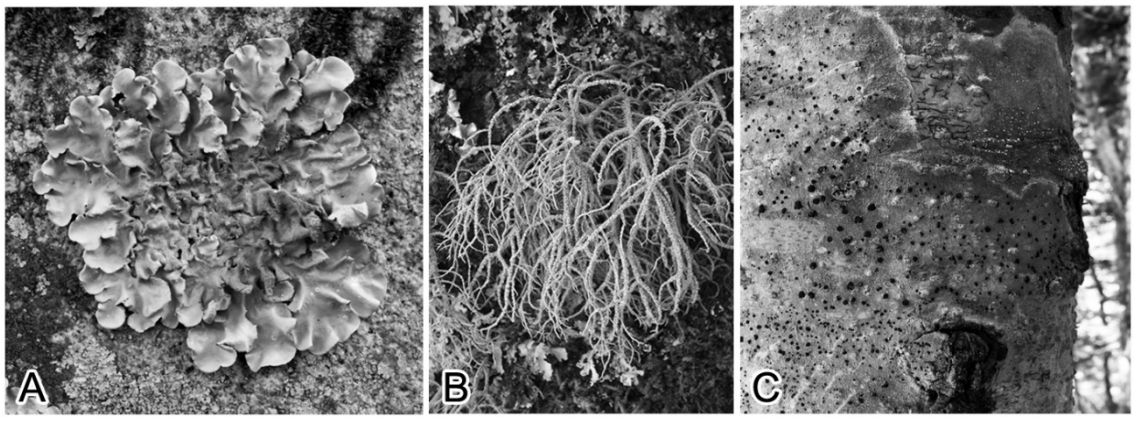


Fig. 1. Growth forms of lichens. A. Foliose (e.g. *Parmotrema tinctorum*). B. Fruticose (e.g. *Usnea rubicunda*). C. Crustose (e.g. *Tephromela atra*).

In Taiwan, there are rich and high diversity of lichens, and a total of ca. 1060 spp. have been reported so far (Aptroot *et al*. 2002, 2003a, 2003b; Aptroot & Sparrius 2003; Kashiwadani *et al*. 2006; Brodo 2007; Coppins *et al*. 2008; Moon & Kashiwadani 2003; Moon *et al*. 2008; Aptroot 2009; Aptroot *et al.* 2009; McCune 2009, 2011; Papong *et al*. 2009; Sparrius *et al*. 2006; Kashiwadani & Moon 2010; Ohmura *et al*. 2010; Ohmura 2012, 2014a). To examine and reveal the nature of lichen diversity, specimens are fundamental materials for the scientific researches.

This article introduces how to collect and make a lichen specimen followed by the methods applied in the lichen herbarium of the National Museum of Nature and Science (TNS), Tsukuba, Japan.

Collection

In order to make "a good specimen of lichen", amount and shape as well as field information are important.

Standard amount for a specimen can be said as "palm size" (ca. 10×10 cm) or more. When the thallus is small in size, many thalli or colonies are usually put together into a packet of specimen. But we have to be careful about contamination in such case. Lichen specimen is used not only for examination of morphology and anatomy but also detection of chemical substances and extraction of DNA. Duplicate specimen is sometimes made, which is divided from the original specimen, for isotype, exsiccata, and other various purpose of researches.

Foliose and fruticose lichens are generally detached from substrates using a knife or picked up by fingers. Foliose lichens should be included both margin and center of thallus, and fruticose lichens should be included from the base to the top of thallus. Crustose lichens are usually collected together with the substrate (Fig. 2). Corticolous crustose lichens, i.e. growing on barks, are collected using a leather craft knife or an army knife. Saxicolous crustose lichens, i.e. growing on rocks, are collected using a hammer and a chisel by breaking the rock (thinner than 2cm thick is much better). When the individual lichen have fruiting bodies (e.g. apothecia and perithecia), they should be included to the collection.

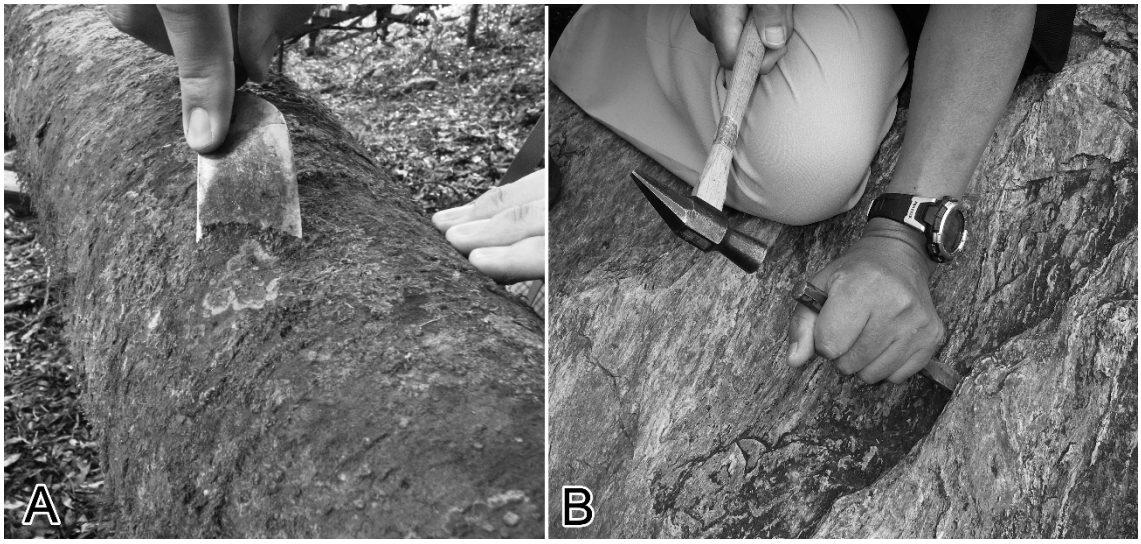


Fig. 2. Collections of crustose lichens. A. Corticolous species collected by a leather craft knife. B. Saxicolous species collected by a hammer and a chisel.

Because the shapes of lichens and the substrates are various as mentioned above, collection tools are also various depending on the purpose and ecological situation. Japanese lichenologists have traditionally used collection tools for lichens as shown in Fig. 3.

Fig. 3. Collection tools. a: paper bags; b: field notebook; c: tweezers; d: secateurs; e:

button-hole cutter; f: leather craft knife; g: chisel; h: hammer; i: armband (or permission); j: hand loupe; k: GPS; l: leather case for secateurs; m: leather case for knife and cutter.

Paper bag/envelope (Fig. 3a). Collection sample is temporary stored in a paper bag/envelope during the field work. Locality number, habitat information, date, and time of photography are noted on the paper. The sizes are, for examples, 185×248 mm (flat type) and 120×70×220 mm (square bottom paper bag). Plastic bag is not good for storage because mold may increase and spoil the collection.

Secateurs (Fig. 3d). This is for cutting a branch or twig.

Button-hole cutter (Fig. 3e). This is useful for the collection of foliose and fruticose lichens growing on rock. The cutter with 12 mm width is preferable.

Leather craft knife (Fig. 3f). Japanese lichenologists have traditionally used this knife since more than one hundred years ago. The knife with 36 mm width is good size for me. It is quite useful and easy for collection of corticolous crustose lichens. However, most lichenologists in other countries outside of Japan use an army knife for collection.

Hammer and chisel (Fig. 3g, h). They are used for breaking a rock. Preferable size of chisel is 13 mm in width and 16 mm in length. Hammer size is depending on personal preference (not too light but not too heavy). The weight of my hammer is ca. 500 g.

Hand loupe (Fig. 3j). Recommended magnification is ×10 to 15. Larger diameter of the lens provides a bright view field. A hand loupe with LED light is also useful especially using in the dark places.

GPS (Fig. 3k). To record the collection site (longitude and latitude). It is better to install a detail map to the GPS instrument.

Leather case for knife, cutter or secateurs (Fig. 3l, m). For the convenience during a field activity and to avoid injury with knife, these cases are useful. It is attached to a waist belt.

Cotton cloth bag. This is for the purpose of packing in the field and transportation. The size is ca. 30×50 cm. The bag has a drawstring at the mouth.

Others. Field notebook, stationery, camera, tripod, tweezers, compass, etc.

Killing insects by storage in a freezer

Some lichens are good habitat and/or feed for larvae, mites, tardigrade, slug, etc. Their eggs may sometimes hutch out within the packet of lichen specimen, by which the cortex and apothecial disc of the specimen are eaten and the amount of insect fecal spoil the specimen. To avoid such accidents, storage in a freezer (at －30℃ for two

weeks) would be effective method for killing insects and their eggs in a lichen specimen.

Making a specimen

The method of making a lichen specimen is different depending on species. 1) Some macrolichens are washed with water and cleaned with removing e.g. mosses. After that, a wet thallus is put between old-newspapers and very weakly pressed between plates (Fig. 4A). Foliose lichens are put on the paper as being flat (Fig. 4B), and fruticose lichens are put as being lateral side (Fig. 4C). The newspaper should be changed every day until the specimens drying up. Heating should not be applied since some lichens having e.g. salazinic acid will turn to red or brown in the thallus color. In addition, spores may eject from the asci. 2) Some tree-barks may strongly vend when they dry. The crustose specimens on such barks should be strongly pressed until they will dry up. 3) Many thalli of small lichens are put together in a small envelope to avoid scattering or dropping from the packet of specimen. 4) Fragments of rocks, soil, barks are sometimes glued on a paper board with arabic gum (Fig. 4D). 5) Specimen of calicioid lichens having fragile stalks are glued on a paper board and protected by a box (Fig. 4E).

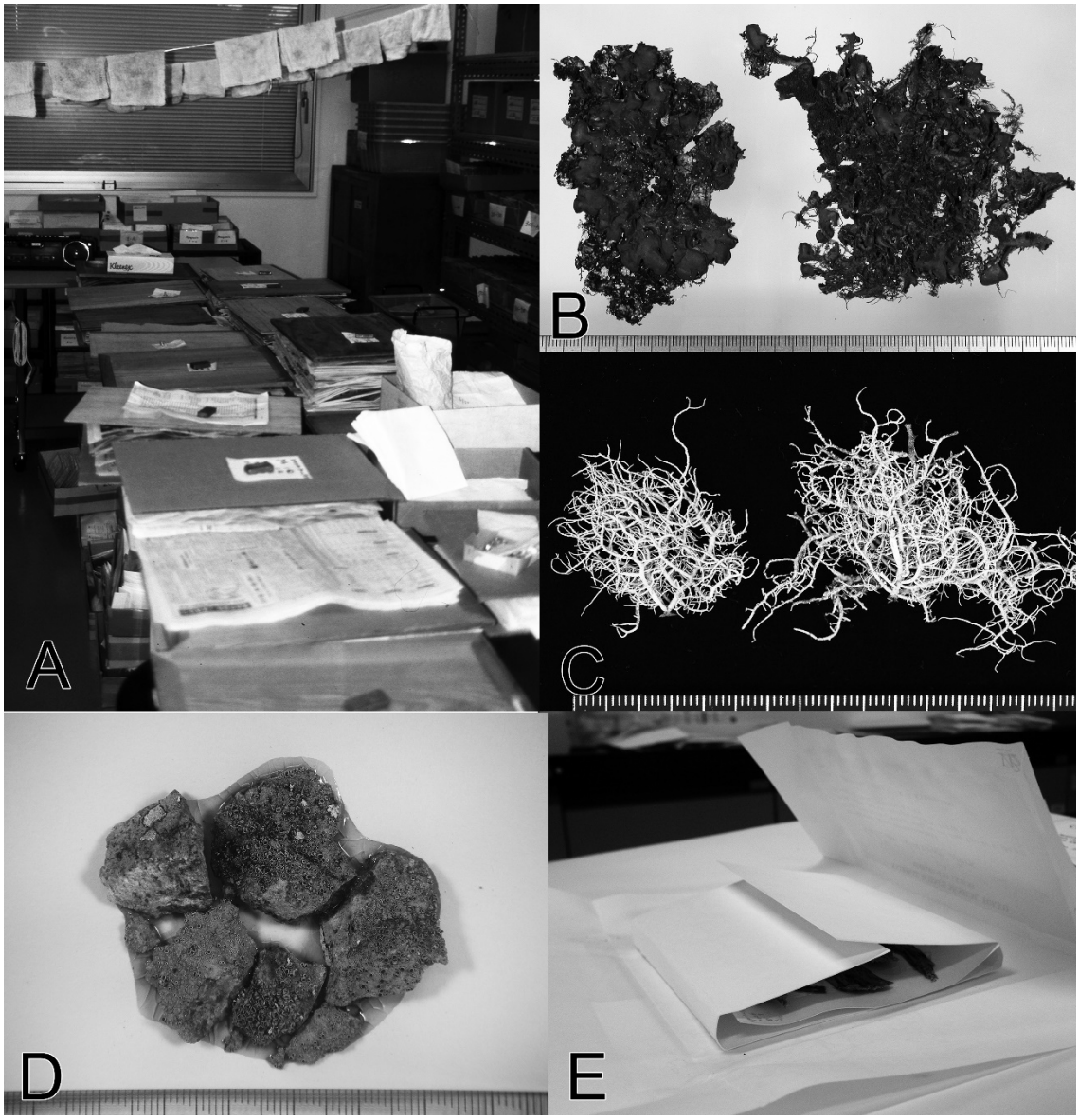


Fig. 4. Making a lichen specimen. A. Washed thalli are put between old newspapers to dry up, and weakly pressed with a weight (ca. 130g). B. Foliose lichen specimen (*Leptogium pseudopapillosm*). C. Fruticose lichen specimen (*Usnea flavocardia*). D. Specimen of saxicolous crustose lichen glued on a paper board. E. Calicioid lichen specimens placed in a paper box.

Specimen packets

Since the size of lichens ranges from a few mm to over several meters, various sizes of specimen packets are convenient. In the lichen herbarium of TNS, five types of packets are used depending on the size of specimen or substrate type (thick paper for rock). The biggest size of specimen packet is about 1/2 page of a newspaper, followed by the size of 1/2, 1/3, 1/6 (Fig. 5).

The specimen packet is glued on a paper sheet (Excel R 220K; 30×43 cm) which

will be placed and arranged in a specimen shelf according to the taxon name and the locality.

Papers for the specimen packet are 1) Spika bond 50K (21×31.5 cm) for a standard specimen, 2) PHO Kent paper 110K (21×31.5 cm) for a specimen on rock, 3) PHO Kent paper 110K (34×31.5 cm), 4) Olympus 108K (36×54 cm), 5) Olympus 108K (45×84 cm). These papers are folded to make a specimen packet as shown in Fig. 5.

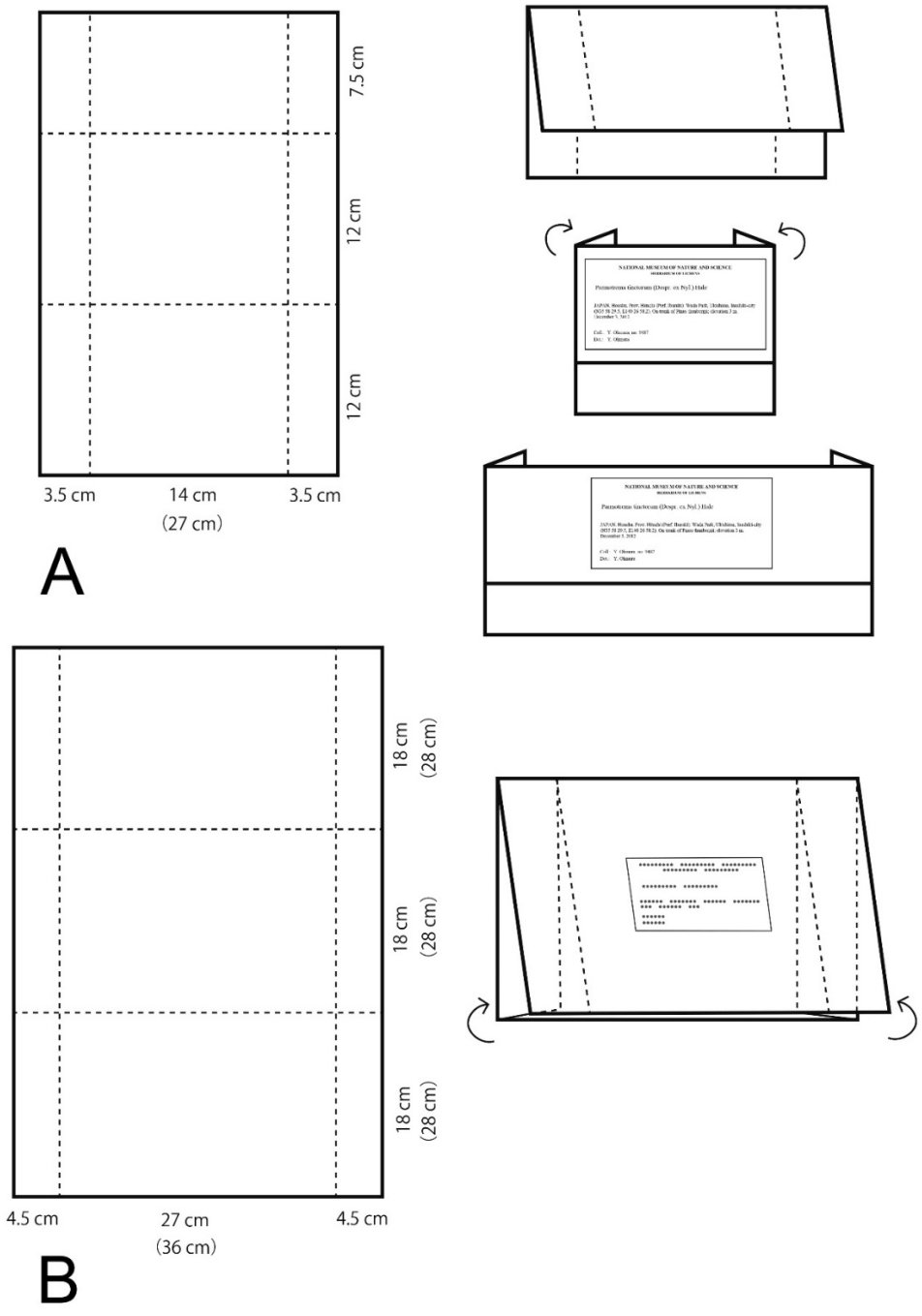


Fig. 5. Preparation of a specimen packet. A. Outside-fold type. First of all, fold the

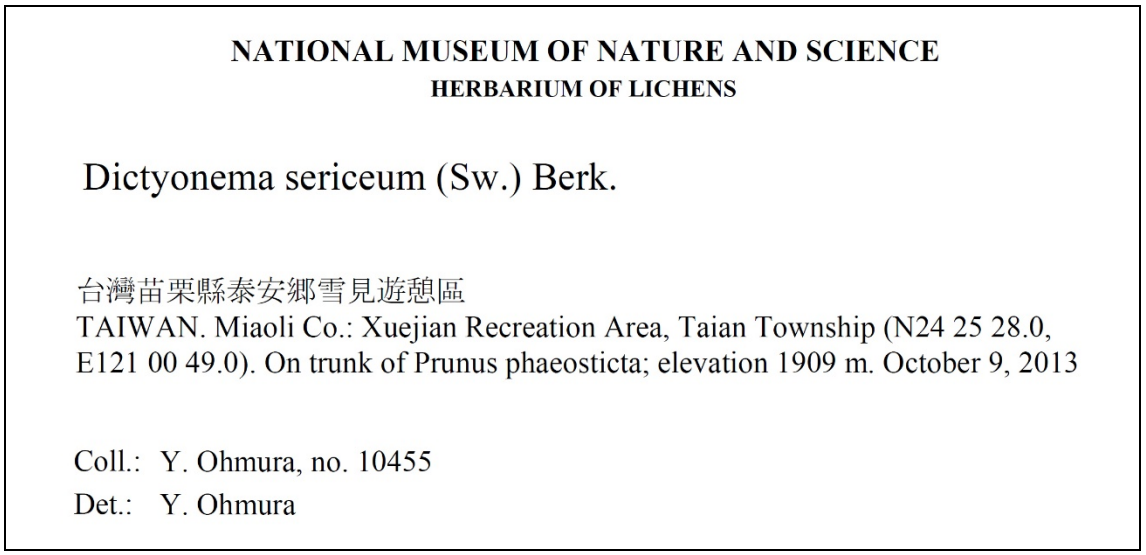
paper in three (12, 12, 7.5 cm), and the both sides (3.5 cm width) are folded outside. B. Inside-fold type. First of all, fold the paper in three (18 cm in each or 28 cm in each). Open a face and then the both side (4.5 cm width) are folded inside, and close the face. Positions of label on the specimen packet are shown in the figure.

Specimen label

A label for a lichen specimen generally includes the following information: title of the specimen, scientific name of the taxon, locality, elevation, substrate, collection date, collector, and identifier (Fig. 6).

Substrate information is mentioned as e.g. “on rock”, “on stone wall”, “on soil”, “on humus”, “on mosses”, “on bark of ...”, “on trunk of ...”, “on twig of ...”, “on branch of ...”, “on leaf of ...”, “on stump”, “on decayed wood”, and “on weathered wood”. Additional information such as vegetation and geographical features are mentioned into this place, as e.g. “on calcareous rock”, “on granite outcrop”, “on rock along a stream”, “on rock of north-facing cliff”, “on bark of *Betula ermanii* in a mixed forest with conifer and broad-leaf trees”, and “over mosses on bark of ...” (Ohmura 2014b).

Language for the label should be written in English. Native language (e.g. Chinese character) for locality name and/or collector can be also added on the label, since the local name is sometime misspelling in English or mispronouncing by a foreign researcher even by a native person. The label is glued on a specimen packet using a brush with starch paste.


NATIONAL MUSEUM OF NATURE AND SCIENCE
HERBARIUM OF LICHENS

Dictyonema sericeum (Sw.) Berk.

台灣苗栗縣泰安鄉雪見遊憩區
TAIWAN. Miaoli Co.: Xuejian Recreation Area, Taian Township (N24 25 28.0, E121 00 49.0). On trunk of Prunus phaeosticta; elevation 1909 m. October 9, 2013

Coll.: Y. Ohmura, no. 10455
Det.: Y. Ohmura

Fig. 6. An example of specimen label made in TNS.

Storage in a herbarium

It is recommended that the air condition in a herbarium should be kept under 50% humidity and under 20℃ at the temperature to protect from attack by insects and molds (Bridson & Forman 1998). In the lichen herbarium of TNS, the temperature is kept under 23℃ and the humidity is under 50%. In addition, fumigation with sulfuryl fluoride for killing insects are conducted once in a year.

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