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# Developing a fast, reproducible, and simple protocol for virtual lichen herbarium using barcoding and QR code techniques

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Abstract: In recent days, biological specimens are digitalized and digital images are available in virtual herbarium for teaching and learning process. Now, there is a need to explore possibilities of usage of barcodes and quick response (QR) codes in developing virtual herbarium for quick access as well as study the taxonomy of repository specimens. In order to establish a virtual herbarium for lichens using barcode and QR code techniques, lichen specimens such as Chrysothrix candelaris (L.) J.R.Laundon, Leucodermia leucomelos (L.) Kalb, Heterodernia flabellate (fee) D.D.Awasthi, Parmotrema andinum (Mull.Arg.) Hale, Parmotrema grayanum (Hue) Hale, Parmellinella stuppeum (Taylor) Hale, and Rampling intermedia (Delise ex Nyl.) Nyl, were collected from the Eastern Ghats and the Western Ghats of Tamil Nadu, India and were identified based on morphological, anatomical and biochemical methods. Moreover, these specimens were preserved in the conventional lichen herbarium as reference materials for future studies. The barcodes and QR codes were generated for all the repository specimens to access the materials as well as to get a complete description of the lichen specimens. The generated barcodes provided the binomial name of lichen specimens along with their accession number. Similarly, the QR codes provided the digital image of lichen specimens along with complete descriptions such as distribution, habit and habitat, growth forms, name of the family, reproductive structure, chemistry, nature of thallus structure and lichen secondary metabolites. From these studies, we standardized a simple, rapid with reproducible protocol to develop a virtual herbarium for lichens to get the digital image and to access the complete descriptions of lichen specimens. This study might be useful for Lichenologists to get information about lichens in digital form and to maintain the lichen wealth for future regenerations without disturbing the lichen biodiversity.

Keywords: Barcodes, conventional lichen herbarium, digital images, lichen biodiversity, QR codes, quick access, repository, specimens.

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# INTRODUCTION

A herbarium is a collection of preserved plant specimens, which are repositories to safeguard plant samples of a given area or region and serve as reference materials to build knowledge of biological resources. Virtual or digital herbaria are playing an important role as a large number of plant specimens are digitized and serve as virtual museums and as libraries of information about plants (Primack et al. 2004). A Virtual herbarium is a digitized form of biological specimens, containing a collection of digital images of preserved plants or plant parts which in turn is useful in improving availability of specimens to all users. In addition, storage of more samples in less space without using herbarium sheet, maintenance of original colour, shape and size of samples without microbial attack and odour emission are the salient features of a virtual herbarium (Flannery 2013). The information on botanical collections is made accessible through digitization, database development and the internet through Barcoding Library (BL) and Quick Response (QR) codes in the digital era.

Barcodes are created in response to the requirement of industries to develop a system to capture the product data quickly during the check-out process at supermarkets. They are one-dimensional optical representations, where widths and spacing of parallel lines are translated primarily into numeric data (Law & So 2010). The information in the barcodes are decoded by electronic devices, linked to a database. There are applications available on the internet to decode the barcode information. Similarly, QR codes are the 2-dimensional barcodes used in the trademark for a type of matrix which has gained recognition as an effective tool for product information. These codes connect digital resources to printed text, suggesting the potential to enhance paper-based learning materials (Chen et al. 2011). It can be read from any direction in 360° through position detection located at the three corners (Moisoiu et al. 2014).

University of Washington Herbarium has developed a virtual database of around 72 genera of lichens. US National Science Foundation along with North American Lichen Herbaria created a virtual database containing 2.3 million North American lichen and bryophyte specimens (Lai 2006). African plants initiative scheme was established in 2013 with the aim of digitizing type specimens and making these images available on the website (Patmore 2010). The American plant systematics created a rich website on Lewis and Clark's botanical collections and Linnean Society's website exhibited plant specimens, insects, fish, and shells in digital form (Reveal 2008). But no efforts were undertaken to create QR codes for repository biological specimens for quick access to get the information about repository specimens especially for lichens.

A digital herbarium is useful to improve the access of potential application and diversity of the lichens which in turn is useful to maintain lichen resources in India. In addition, lichens are slow growers and require several years to develop thallus to the length of 1 cm (Ahmadjian 1993). Keeping this in mind, studies were undertaken to create a virtual herbarium for lichens using digital picturization, barcoding and QR code techniques in cloud environment. A simple and rapid protocol was standardized to create virtual herbarium for lichens and subsequently made available online for Lichenologists.

## MATERIALS AND METHODS

# Collection and identification of lichen samples

Lichen samples were collected from various living and non-living substrates in the Eastern Ghats (Kolli & Yercaud hills) and the Western Ghats (Kodaikanal & Nilgiris hills) of Tamil Nadu, India and were identified by following the standard method of Awasthi (2007). Lichen morphology, anatomy, growth forms, powdery appearance and nature of fruiting bodies embedded on the thallus were critically analysed to identify the lichen communities from genus to species level. Chemical tests (K, C, KC, and PD) were employed to observe the colour reactions on lichen thallus including the existence of lichen secondary metabolites. Lichen thallus were examined for the cortical and medullary chemical compounds by thin layer chromatography method using a suitable solvent system (Orange et al. 2001). The specimens were deposited in the Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India as per the conventional method.

# Digitization and preparation of Barcoding for lichen specimens

The collected lichen thalli were placed in an Image capturing documentation system fitted with a high resolution digital camera (Precision Co, Ltd, India) to capture the overall images of lichens without any background noise error to minimise the pixel size. Lichen images were also directly photographed using high resolution digital cameras or smartphones with different dimensions on the substratum without disturbing the lichen biodiversity. Lichen images were taken to observe specific parts such as isidia, rhizines, apothesia, soredia etc to understand the digitalized herbarium (https:// www.digitallichenbu.in/).

Selected images of lichens with smaller pixel size were transferred to a computer terminal installed with a barcode generator software studio containing RFID label software (TBarCode SDK Activator<sup>®</sup>) to generate barcodes (Ginni et al. 2022). The barcoding data was generated with individual bars along with numeric numbers without any decimal for lichen specimens such as Chrysothrix candelaris (L.) J.R.Laundon (BU/ BRL/2022/002), Leucodermia leucomelos (L.) Kalb (BU/BRL/2022/022), Heterodernia flabellate (fee) D.D.Awasthi BU/BRL/2022/012), Parmotrema andinum (Mull.Arg.) Hale (BU/BRL/2022/024), Parmotrema grayanum (Hue) Hale (BU/BRL/2022/025), Parmellinella stuppeum (Taylor) Hale (BU/BRL/2022/031), and Ramalina intermedia (Delise ex Nyl.) Nyl. (BU/ BRL/2022/036).

# Digitization and preparation of QR codes for lichen specimens

In order to create QR codes for each lichen specimen, QR code generator software studio containing MacOSX. pkg. 10.8+Version: 1.0.3 software was used and then processed digitally so as to read the contents rapidly. Attempts were made to read QR codes in both windows PC and mobile phone devices. If the mobile device did not build in any QR code reader, the user needs to download the right decoder from google play store and install it on to the device. The generated image files as QR codes were used to identify the lichen specimens from genus to species level along with detailed descriptions such as distribution, habit and habitat, family, nature of thallus, reproductive structure, chemistry (colour tests) and secondary metabolites of each lichen sample and were documented (Diazgranados & Funk 2013).

# RESULTS

Barcodes and QR codes empowered virtual herbarium for lichens was created wherein, the virtual data was made available online. The virtual data such as digital image, name, and descriptions of lichens were presented. Digital picturization as virtual data for lichen identification can be accessed by the end users of both Windows PCs and smartphone mobile devices online using both barcode and QR code techniques. The end users need to download the right decoder software from google play store and install it on to the device for QR codes. On the other hand, a barcode scanner is necessary to scan the barcode to read the data. The barcodes were generated and displayed in the conventional lichen herbarium in Bharathiar University, Coimbatore, India to get the details of particular lichen specimens. If we scan the barcodes, the binomial name of lichen specimens and their accession numbers are displayed.

Lichen specimens such as Chrysothrix candelaris (L.) J.R.Laundon, belonging to crustose, Leucodermia leucomelos (L.) Kalb, Heterodernia flabellate (fee) D.D.Awasthi, Parmotrema andinum (Mull.Arg.) Hale, Parmotrema grayanum (Hue) Hale, Parmellinella stuppeum (Taylor) Hale belonging to foliose and Ramalina intermedia (Delise ex Nyl.) Nyl. belonging to fruticose growth forms were identified from genus to species level (Image 1). Different genus and groups of the lichens name was given different code to predict the digital herbarium sample. These repository specimens have been deposited in the lichen herbarium as reference materials as per the conventional method of preparation for future taxonomic studies (Image 2). By using the barcode generator software studio, barcodes were generated and labelled properly for each repository specimen along with their accession numbers (Table 1).

According to Table 2, QR codes were created for all of the chosen lichen species and they provide a brief description that includes information on distribution, habit & habitat, growth forms, name of the family, reproductive structure, chemistry (colour tests), nature of thallus structure, and existence of secondary metabolites. Along with detailed descriptions of each lichen specimen, a digital image is also displayed on the screen. A simple, reliable with reproducible protocol was developed to identify the repository specimens using QR code reader significantly (Image 3). Barcode and QR approaches reveal easy identification and prediction of lichen images very fast with a complete description.

## DISCUSSION

Lichenologists identify lichen species routinely by their external and internal morphology along with chemical constituents contained in thallus and to some extent to molecular traits by means of DNA profile (Upreti et al. 2005). Lichen taxonomy is a very complex and time-consuming process that also suffers from shortage of skilled manpower (Nayaka & Upreti 2013). A large number of lichens are being preserved

	Barcode	Binomial name	Accession number
1	9782022002	Chrysothrix candelaris (L.) J.R.Laundon	BU/BRL/2022/002
2	9782022022	Leucodermia leucomelos (L.) Kalb	BU/BRL/2022/022
3	9782022012	<i>Heterodernia flabellate</i> (fee) D.D.Awasthi	BU/BRL/2022/012
4	9782022024	Parmotrema andinum (Mull.Arg.) Hale	BU/BRL/2022/024
5	9782022025	Parmotrema grayanum (Hue) Hale	BU/BRL/2022/025
6	9782022031	Parmellinella stuppeum (Taylor) Hale	BU/BRL/2022/031
7	9782022036	Ramalina intermedia (Delise ex Nyl.) Nyl.	BU/BRL/2022/036

Table 1. Barcoding for lichen specimens and the accession number.

using conventional herbarium methods for a variety of research and teaching and learning purposes in the world. But to the best of our knowledge, no attempt has been made so far for developing a virtual herbarium for lichens using digital picturization, barcoding library and QR code techniques in India. The present study was developed to establish a virtual herbarium for lichens with a simple, reliable and user-friendly protocol (Image 3). For a few specimens a barcode library and QR code information virtual data were developed and made available on the website https://www.digitallichenbu. in/ (Tables 1 & 2).

Both barcodes and QR codes showed brief information about the lichen characteristic features in a machine-readable optical label structure. It is used extensively in research for barcoding of flora and fauna in the digital world. Each barcode image is programmed to identify the name of the plant and other information relevant to the plant family, order and taxonomical description. A large number of benefits of QR codes and barcoding system have been listed out (Chase & Fay 2009) like improved inventory management,



Image 1. Lichen species used for creating lichen virtual herbarium: A—*Chrysothrix candelaris* (L.) J.R.Laundon | B—*Leucodermia leucomelos* (L.) Kalb | C—*Heterodernia flabellate* (fee) D.D.Awasthi | D—*Parmotrema andinum* (Mull.Arg.) Hale | E—*Parmotrema grayanum* (Hue) Hale | F—*Parmellinella stuppeum* (Taylor) Hale | G—*Ramalina intermedia* (Delise ex Nyl.) Nyl. © https://www.digitallichenbu.in/

faster check-in and check-out facility, easy to sort out the specimens, reduced staff workload and skilled man power and increased accuracy and efficiency (Singh 2016).

Virtual herbarium of angio-spermic plants of the Western Ghats of Maharashtra, India, is available with the Modern College of Arts, Science and Commerce, Pune, Maharashtra, India, in which a list of about 1,000 species was made, of which 650 plants were documented and the data on 350 plants is currently available on the website (Singh & Sharma 2009). The primary objective of this project was to capture and store high quality digital images of plant species and to make this database available to students, researchers, and public to disseminate the awareness of regional plants (http://www.indianflora.org/). A virtual herbarium for the higher plants has been created at the Kerala Forest

Research Institute, Peechi, Kerala, India, which provides a total of 5,718 records representing 203 plant families and is rendered accessible at http://kfriherbarium.org/ (Sreekumar et al. 2017). Similarly, a digital herbarium for the flora of Karnataka was carried out by Rao et al. (2012) at the Indian Institute of Science, Bangalore, India.

The high-resolution images of digitized plant specimens through virtual herbarium techniques may be useful to examine micro-morphological features of plant parts and can further access the repository specimen information recorded on the data sheet. In addition, using barcodes, plant specimens could be sorted out based on family and order as per the classification with more accuracy and efficiency in the virtual herbarium (Dmitry et al. 2017). It is reported that virtual lichen herbarium is less time-consuming and needs fewer

# Table 2. QR codes of lichen specimens and their brief descriptions.

	Lichen species	QR code	Descriptions of lichen species
1.	Chrysothrix candelaris (L.) J.R.Laundon		<ul> <li>ID: BU/BRL/2022/002</li> <li>Distribution: Eastern and Western Ghats of Tamil Nadu, India</li> <li>Habitat: Found in Angiosperms tree barks</li> <li>Ecology: Open habitats and attached with substratum</li> <li>Family: Parmeliaceae</li> <li>Thallus: Crustose, leprose, unstratified or, in thick specimens, sometimes indistinctly stratified, indeterminate, thin, irregularly spreading, sometimes forming scattered granules, but usually ±continuous</li> <li>Upper surface: Bright yellow throughout, often with an orange or greenish tinge, composed of a mass of fine soredia, 12–30(-40) µm in diam.</li> <li>Medulla: Usually not evident, in thick thalli sometimes indistinctly present, yellow</li> <li>Apothecia: Ascomata, up to 0.5 mm in diam., ±superficial disc: pale orange, often yellow-pruinose margin: thin, ecorticate, soon becoming excluded exciple: poorly developed, composed of a nastomosing hyphae epihymenium: hyaline, up to 18 µm tall, composed of of a reticulate layer of richly branched paraphysoids</li> <li>hymenium: hyaline, up to 50 µm tall (including epihymenium); paraphysoides: 1–1.5 µm wide, richly intertwined in epihymenium; hypothecium: colorless, poorly developed</li> <li>Ascospores: Asci: Clavate, 8-spored ascospores: 9–14 x 3 µm</li> <li>Pycnidia: Not observed</li> <li>Reproductive Structure: Apothecia</li> <li>Chemistry: K- or K+ Orange, sometimes darkening to red-black, C-, KC-, P- or P+ orange; UV+ dull orange or UV-</li> </ul>
2.	Leucodermia leucomelos (L.) Kalb		Secondary metabolites: calycin and/or pinastric acid         ID: BU/BRL/2022/022         Distribution: Eastern and Western Ghats of Tamil Nadu, India         Habitat: Found in both Gymnosperms and Angiosperms tree barks, rocks and soil         Ecology: Open habitats and loosely with substratum         Family: Physciaceae         Thallus: Foliose type, pendulous and covering large areas, corticolous or terricolous         Lobes: dichotomously branched, ascending, tapering at apices         Upper surface: Canaliculated, pinkish-brown, erhizinate         Soredia: Common at apices         Medulla: White         Apothecia: Rare, sub terminal,         Ascospores: Ellipsoid, 8 spores,         Reproductive Structure: Soredia         Chemistry: Cortex K* yellow, C, KC, P*; Medulla K* or K* (yellow to red), C, KC, Por P* (red); TLC method         detected sekikaic acid, zeorin, chloroatranorin, zorsticitc acid and salazinic acid
3.	<i>Heterodernia flabellate</i> (fee) D.D.Awasthi		<ul> <li>ID : BU/BRL/2022/012</li> <li>Distribution: Eastern and Western Ghats of Tamil Nadu, India</li> <li>Habitat: Found in both Gymnosperms and Angiosperms tree barks</li> <li>Ecology: Open habitats and loosely with substratum</li> <li>Family: Physciaceae</li> <li>Thallus: Foliose to sub fruticose, often in loose rosettes or forming tangled mats, loosely adnate or, in part, unattached, 5–15 cm wide</li> <li>Lobes: 0.7–2.5 mm wide, ca. 2–4 mm wide at the tips, plane to weakly convex, sublinear to linear-elongate, regularly to irregularly branched, radiating; apices not ascending, contiguous to discrete, with short lateral lobes</li> <li>Upper surface: Gray-white to greenish-white, ±partly blackened in the center.</li> <li>Lower surface:</li> <li>Soredia: lacking Soredia, Isidia, and Pruina</li> <li>Medulla: White, lower medulla dark yellow to orange-brown</li> <li>Apothecia: Common, laminal, sessile to sub stipitate, 1–6 mm wide; margin crenate at first, lobulate at maturity; inner surface of lobules ecorticate, yellow-orange pigmented; disc concave, dark brown to brown-black, epruinose or weakly white pruinose</li> <li>Ascospores: Polyblastidia-type, ellipsoidal, with 2–3 sporoblastidia present at maturity, 27–40 × 12–19 µm. Pycnidia common, immersed, then becoming emergent, visible as black dots; conidia bacilliform, 4–5 × 1 µm</li> <li>Reproductive Structure: Apothecia</li> <li>Chemistry: Cortex K+ (yellow), C–, KC–, P+ yellow; upper medulla K+ yellow, C–, P–; lower surface K+ violet;</li> <li>Secondary metabolites: atranorin (major), zeorin (major), 16β-acetoxyhopane-6α,22- diol (major), leucotylin (minor), 7- chloroemdin (minor), flavoobscurins A, B1, B2 (minor)</li> </ul>

Preethí 5 Ponmurugan

	Lichen species	QR code	Descriptions of lichen species
4.	Parmotrema andinum (Mull.Arg.) Hale		<ul> <li>ID: BU/BRL/2022/024</li> <li>Distribution: Eastern and Western Ghats of Tamil Nadu, India</li> <li>Habitat: Found in both Gymnosperms and Angiosperms tree barks</li> <li>Locality: Yercaud hills of Eastern Ghats (Altitude 1515 meters above MSL height, 11.7211° N &amp; 78.1835° E)</li> <li>Ecology: Open habitats and loosely with substratum</li> <li>Family: Parmeliaceae</li> <li>Thallus: Foliose, loosely attached to the substratum.</li> <li>Lobes: Lobes ascending, rotund, up to 5–10 mm wide, 120–180 µm thick; margin crenate, eciliate</li> <li>Upper surface: Black, slightly wrinkled, with 3–5 mm wide, erhizinate marginal zone. Rhizines in the center, simple, short up to 1mm long</li> <li>Medulla: White, 100–120 µm thick</li> <li>Apothecia: Rare, Stipitate, up to 10 mm in diameter, disc brown, amphithecium rugose, maculate, epithecium brown, 15–20 µm thick; hymenium 55–65 µm high. Asci clavate, 8-spored, 30–45 x 19 µm</li> <li>Ascospores: Spores colourless, simple, ellipsoid, 14–22 x 7–10 µm. Pycnidia laminal, towards apices, black. Conidia filiform, 10–15 µm. Long.</li> <li>Reproductive Structure: Apothecia</li> <li>Chemistry: Cortex K* (yellow), yellow: medulla K-, C+ red, KC + red, P-</li> <li>Secondary metabolites: Leconoric acid</li> </ul>
5.	Parmotrema grayanum (Hue) Hale		ID: BU/BRL/2022/025 Distribution: Eastern and Western Ghats of Tamil Nadu, India Habitat: Found in Angiosperms tree barks and in rocks. Ecology: Open habitats and loosely with substratum Family: Parmeliaceae Thallus: Foliose type, Saxicolous Lobes: Rotund to irregular; margins Upper surface: Pale grey to grey green, shiny, becoming dull towards the thallus center, somewhat longitudinally folded in the marginal region and emaculate, granular to filiform, simple to coralloid, branched, thin, brown tipped or concolorous Lower surface: Black, minutely wrinkled, smooth, shiny, with a broad, erhizinate, pale brown to dark tan marginal zone; rhizines sparse, simple with short. Soredia: Abundantly sorediate; soralia marginal, linear to labriform (± crescent-shaped), or subcapitate; soredia ± granular, typically discolored by a dark gray tinge, pale inside Medulla: White Apothecia: Not seen Reproductive Structure: Isidia Chemistry: Cortex P+ yellow, K+ yellow, KC-, C-, UV-; medulla P-, K-, KC-, C-, UV- Secondary metabolites: Atranorin, and protolichesterinic acid
6.	Parmellinella stuppeum (Taylor) Hale		<ul> <li>ID: BU/BRL/2022/031</li> <li>Distribution: Eastern and Western Ghats of Tamil Nadu, India</li> <li>Habitat: Found in both Gymnosperms and Angiosperms tree barks</li> <li>Ecology: Open habitats and loosely with substratum</li> <li>Family: Parmeliaceae</li> <li>Thallus: Foliose, adnate to loosely adnate, 2–20 cm in diam., lobate.</li> <li>Lobes: Sub irregular, elongate, slightly imbricate, plane, separate, 4–8 mm wide; apices: rotund, ciliate; cilia: up to 2.0 mm long.</li> <li>Upper surface: Gray, smooth, dull, emaculate</li> <li>Lower surface: black with brown, naked zone peripherally, centrally rhizinate; rhizines: scattered, simple, black</li> <li>Soralia: Granular, common, in linear to orbicular, laminal or marginal soralia</li> <li>Medulla: White with continuous algal layer</li> <li>Apothecia: Rare, Sub stipitate, up to 30 mm in diam.; margin: crenulate; disc: brown, imperforate.</li> <li>Ascospores: Ellipsoid, 12–17 x 6–9 µm Pycnidia: common, punctiform conidia: sublageniform, 4–6 x 1 µm</li> <li>Reproductive Structure: Soralia, Ascospores</li> <li>Chemistry: Upper Cortex K+ yellow, C-, KC-, P-; medulla K+ yellow turning deep red, C-, KC-, P+ orange.</li> <li>Secondary metabolites: atranorin, chloroatranorin, salazinic acid and consalazinic acids (minor).</li> </ul>
7.	<i>Ramalina intermedia</i> (Delise ex Nyl.) Nyl		ID: BU/BRL/2022/036 Distribution: Eastern and Western Ghats of Tamil Nadu, India Habitat: Found in both Gymnosperms and Angiosperms tree barks Ecology: Open habitats and hanging from substratum Family: Ramalinaceae Thallus: Fruticose, caespitose, up to 3 cm long Lobes: Sparingly branched from a narrow holdfast branches: flat, +dorsiventral or subcylindrical, irregular in thickness in cross section, tips often ending in soralia, up to 1.5 mm wide Upper surface: Greenish or gray, rarely canaliculated and smooth Lower surface: Thin; chondroid strands: continuous, cracked Soralia: subterminal or marginal soralia that are 0.4–0.5 mm in diam. Pseudocyphellae: Occasional Medulla: White Apothecia: Not observed Reproductive Structure: Soralia Chemistry: Cortex K-, C-, KC+ yellow, P-; medulla K-, C-, KC-, P- Secondary metabolites: usnic acid, homosekikaic acid, sekikaic acid, 4'-O-methylnorhomosekikaic acid (minor).



Image 2. Conventional lichen herbarium exhibiting barcodes and QR codes.

skilled manpower; also explores the bioactive properties of lichen genera for industrial applications (Flannery 2013). A digital lichen herbarium might be useful to researchers to easily access the lichens of the specific herbaria for their studies.

To conclude, lichen specimens such as *Chrysothrix candelaris* (L.) J.R.Laundon, *Leucodermia leucomelos* (L.) Kalb, *Heterodernia flabellate* (fee) D.D.Awasthi, *Parmotrema andinum* (Mull.Arg.) Hale, *Parmotrema grayanum* (Hue) Hale, *Parmellinella stuppeum* (Taylor) Hale, and *Ramalina intermedia* (Delise ex Nyl.) Nyl. collected from the Eastern Ghats and the Western Ghats of Tamil Nadu, India were digitalized for making a virtual herbarium. The barcodes and quick response (QR) codes were used in the virtual lichen herbarium for quick access and to get a complete description of the repository specimens based on morphological, anatomical and biochemical characterization traits. The present attempt may be highly useful to lichenologists and biodiversity conservation scientists to get information about lichens in digital form without disturbing the lichen biodiversity in the habitats.

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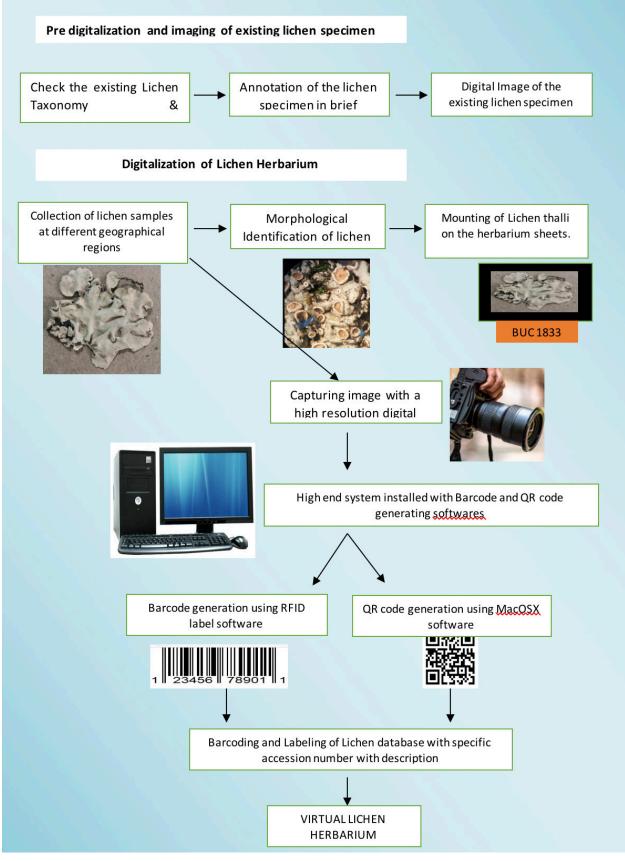


Image 3. Steps involved in creating a virtual herbarium for lichens using barcode and QR code techniques.



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