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Research Article

Characterisation of Fruticose Lichen Genus *Stereocaulon* from Sabah Based on Morphology, Chemotyping, and Molecular Typing

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ABSTRACT

Lichens in the genus *Stereocaulon* are commonly found in high-elevation mountains in tropical regions. Taxonomy of *Stereocaulon* is always an ongoing topic discussed among lichenologists, especially for species-level identification due to polymorphism and chemical diversity of *Stereocaulon*. In this study, we examined *Stereocaulon* spp. from Mount Kinabalu, the highest mountain in Borneo (4095 m). A total of 42 *Stereocaulon* specimens were included in this study; 40 were newly collected between 1900 m and 3700 m a.s.l. along the summit trail of Mount Kinabalu, and two specimens from Mount Alab (1900 m) were previously published. We used integrative approaches to delimit the specimens into Operational Taxonomic Units (OTUs) based on macro-morphology, chemical profiles, and molecular phylogeny. Macro-morphological characters, including apothecia (reproductive organ), phyllocladia, pseudopodetia and cephalodia were examined, while chemical profiles were obtained from spot tests and High-performance thin-layer chromatography (HPTLC). A phylogenetic tree was constructed based on the ITS gene by using Bayesian and Maximum Likelihood analyses. A total of six Operational Taxonomic Units (OTUs) were identified and were provisionally named according to their diagnostic morphological and chemical characters, namely, RF (ramular-fluorescence), RN (ramular-nonfluorescence), GF (granular-fluorescence), GN (granular-nonfluorescence), RU1 (ramular-curved 1) and RU2 (ramular-curved 2). The monophyly of each of the OTUs is supported by phylogenetic analysis. Due to lack of reliable identification keys and reference genetic data for this genus, we could not determine the species identities for the OTUs revealed in this study. Nevertheless, the findings of this study provide a baseline for future studies on the taxonomy of *Stereocaulon* species in Mount Kinabalu, integrating morphology, chemistry, and genetics. The implementation of high-performance thin-layer chromatography (HPTLC) profiles show potential for distinguishing samples. However, further research with more *Stereocaulon* samples from different taxa and regions is necessary to verify the reliability of this method.

Keywords: Snow lichen; integrative taxonomy; alpine ecosystem; montane forest; thin-layer chromatography; high-performance thin-layer chromatography (HPTLC); mountain ecosystem; Borneo.

INTRODUCTION

Genus *Stereocaulon* is a widely distributed fruticose lichen genus. These lichens grow mostly in upland regions on siliceous rock, particularly on recent volcanic rock, on metal-rich spoil heaps, and on acidic soil among mosses (Oset, 2014; Ismed et al., 2018). There are around 140 species of *Stereocaulon*, including varieties and forms of the same species, that have been recorded worldwide (Kirk, 2001; Oset, 2014). For *Stereocaulon*, the key morphological characters that have been used for species-level identification are pseudopodetia, phyllocladia, cephalodia, apothecia, and the shape and number of microspores (Fries, 1858; Lamb, 1978; Oset, 2014; Park et al., 2018). Some of the key morphological characters show polymorphism due to the rock types, elevations, and environmental factors, which include light intensity, humidity, air quality and chemical composition of the surroundings (Lamb, 1977; Huang, 2010; Oset, 2014; Athukorala et al. 2016; Park et al., 2018; Lõhmus et al. 2023; McCune et al., 2023). This high polymorphism has made species identification difficult (Myllys et al., 2001, Orange et al., 2001; Oset, 2014). Hence, chemical profiling and phylogenetic approaches, in addition to the morphological characters, have been used to improve the taxonomy of *Stereocaulon* (Lamb, 1951, 1977, 1978; Tønsberg, 1977; Orange et al., 2001; Huang, 2008, 2010; Huyen et al., 2017; McCune et al., 2023; Torres et al., 2023).

The first comprehensive worldwide review of *Stereocaulon* was conducted by Lamb (1951, 1977, 1978), which provided identification keys based on morphological and chemical profile data, including spot tests and thin-layer chromatography. There are very few studies that investigate the detailed chemical composition of *Stereocaulon* (Tønsberg, 1977). Currently, chemical profiling is an essential part of *Stereocaulon* taxonomy and overall lichen taxonomy, especially for species-level identification (Lamb, 1978; Tønsberg, 1977; Orange et al., 2001; Oset, 2014; Huyen et al., 2017; Park et al., 2018; Torres et al., 2023). The commonly used method for the taxonomy of lichen is thin layer chromatography (TLC) and frequently used solvent systems for development include Toluene:Dioxane:Acetic acid (180:45:5 v/v/v), Hexane:Methyl ter-butyl ether:Formic acid (140:72:18v/v/v), and Toluene:Acetic acid (170:30 v/v), which have improved the taxonomic classification (Orange et al., 2001). Atranorin, lobaric acid, stictic acid and norstictic acid are common compounds found in the genus *Stereocaulon* (Lamb, 1978; Tønsberg, 1977; Orange et al., 2001; Oset, 2014; Park et al., 2018).

Due to the high morphological and chemical polymorphism of *Stereocaulon* species, genetic sequences of selected genes have been used to resolve taxonomy problems, both in terms of classification and identification. Many genera and species of lichen have been re-identified and reclassified over the years due to the introduction of genetic analysis (Huang, 2008; Leavitt et al., 2013; Oset, 2014 Park et al., 2018). Many species have a wide distribution across different continents and large geographical areas, but comprehensive studies comparing their morphological and chemical profiles across their full range are rare. This makes it difficult to establish reliable references for species identification (Huang, 2010; Huyen et al., 2017).

In Malaysia, the records of *Stereocaulon* are from the high-elevation habitats on mountains. To date, six taxa that were recorded from Mount Kinabalu, Sabah, which include *S. graminosum* Schaer., *S. granulans* Sipman, *S. halei* I.M. Lamb, *S. massartianum* Hue, *S. massartianum* var. *chlorocarpoides* (Zahlbr.) I.M. Lamb, and *S. staufferi* var. *borneense* I.M. Lamb (Sipman, 1993). Previous studies have shown that these species occupy different elevational zones, and that dominance of the foliose lichens at higher elevations is due to higher humidity and higher light intensity (Sipman, 1993; Huang, 2008, 2010; Hyde et al., 2023). In this study, we used integrative approaches to delimit *Stereocaulon* spp. from Mount Kinabalu

into Operational Taxonomic Units (OTUs) based on macro-morphology, chemical profiles, and molecular phylogeny. We also discussed the possible species identity of the OTUs based on available references for macro-morphology and chemical profiles from the literature. Our study will provide information for this region about the morphological, chemical and genetic variations.

METHODOLOGY

The study site and collection of *Stereocaulon* lichens

This research was primarily conducted at Mount Kinabalu (4096 meters above sea level, hereinafter a.s.l.), Sabah, Malaysia. Transect sampling was conducted on the mountain climbing route (Summit Trail) from the Sabah Parks Kinabalu headquarters (1500 m a.s.l.) to the peak (4096 m a.s.l.) (Fig. 1). In addition, two *Stereocaulon* specimens from Mount Alab, collected from the sampling conducted between 1200 m a.s.l. and 1900 m a.s.l. (as previously described in Lim, 2019), were also incorporated in this study. *Stereocaulon* can be easily seen in the field due to their white colour and prominent short-stalked structure (secondary thallus with pseudopodetia), which can only be found growing in large clumps on substrates such as soil and rock in areas with high light intensity (usually open areas with direct sunlight) (Oset, 2014).

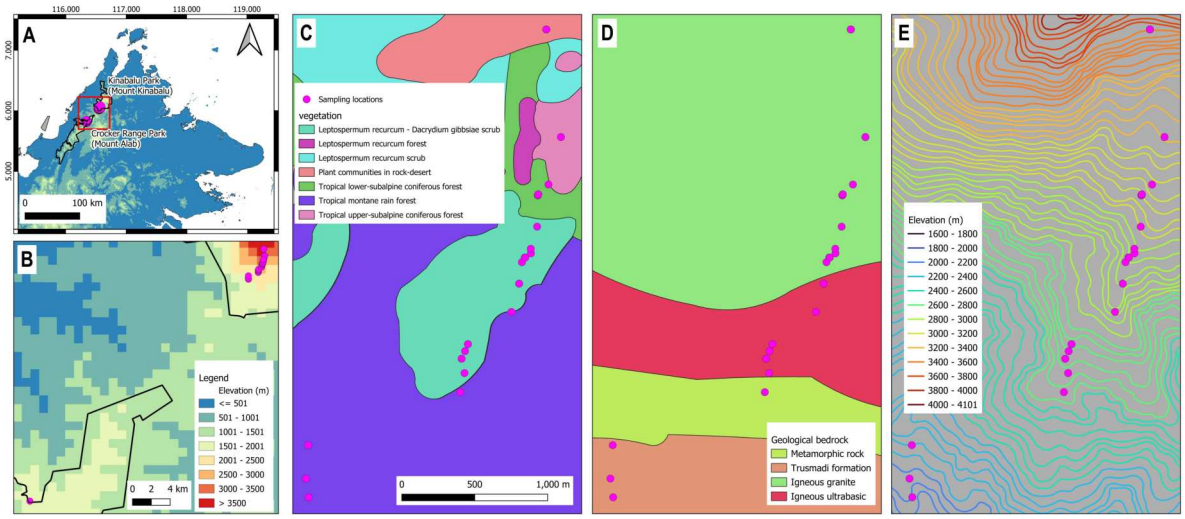

Figure 1: Study area and sampling locations on Mount Kinabalu and Mount Alab, Sabah. **A.** Overview map of Sabah showing Kinabalu Park and Crocker Range Park as the study area (red box). **B.** Enlarged map of the study area within Kinabalu Park and Crocker Range Park showing sampling locations (pink dots). **C.** Vegetation map of the summit trail area with sampling locations on Mount Kinabalu; source for vegetation type: Kitayama (1991). **D.** Geological map of the summit trail area with sampling locations on Mount Kinabalu; source for geological type: Badang (1999). **E.** Topographic map of the summit trail area showing detailed elevation contours and sampling locations.

For each population (the clump) of *Stereocaulon* that was observed in the field, habitat information like soil type, elevation, and vegetation type around the lichen population was recorded. Then, a sample of a 10 cm × 10 cm small patch of lichen, each consisting of around 100 g of fresh specimen, was collected by using a small shovel at each of the 42 locations along the Summit Trail (Fig. 1). The samples were immediately processed in the field by removing debris and contamination, and sorting them into three subsamples: one for morphological

examinations, one for genetic sequencing, and one for chemical analysis. The subsample for DNA analysis was kept in a separate tea bag and stored in air-tight containers filled with silica gel in the field (Din et al., 2010; Oset, 2014). Upon returning from the field, the containers with specimens for genetic isolation were stored in a –80°C freezer for further analyses. The specimens for morphological and chemical analysis were air-dried and then were kept in paper bags which were then stored in containers with silica gel. All samples were deposited at BORNEENSIS Herbarium (BORH) of Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah with accession codes: BORH 5854 – BORH 5898 (Table 1).

Morphological examination of the collected *Stereocaulon* lichens

Upon return to the laboratory, the morphological characteristics of all the collected specimens were examined using a stereo microscope (Olympus SZ61, .0.67× – 4.5×) and a compound microscope (Olympus CX23). The key characters of *Stereocaulon*, namely, pseudopodetia (C-shape – curved vs. Y-shape – branched, Fig. 2A and Fig. 2B), location of apothecia (Lateral – alongside the lichen body vs. Terminal – at the tip of the lichen body, Fig. 2C and Fig. 2D), phyllocladia (Ramular or Coral-like vs. Granular or Clumpy, Fig. 2E and Fig. 2F), and cephalodia (location of cyanobacteria) were examined, measured, and photographed. Based on these morphological characters, we assigned the samples into 4 morphological groups as shown in Fig. 2. Both the identification of these key morphological characters and for determination of the possible species identity of the OTUs were done based on Sipman (1993), as it provides the most recent record with morphological descriptions of *Stereocaulon* species from the region.

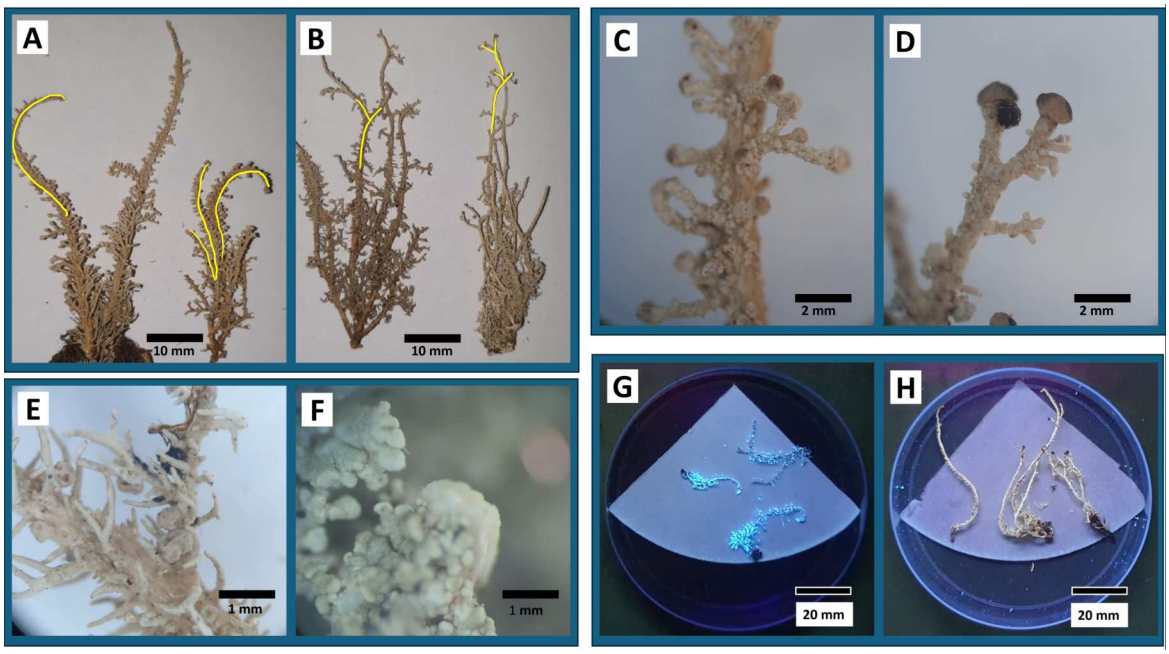

Figure 2: Morphological characteristics of the lichen *Stereocaulon* from Mount Kinabalu, Sabah. Pseudopodetia: **A.** (1) C-shape / Curved shown **B.** (2) Y-shape / Branched shown **C.** Location of apothecia: (1) Lateral / Alongside the lichen body **D.** (2) Terminal / At the tip of the lichen body **E.** Type of phyllocladia: (1) Ramular / Coral-like **F.** (2) Granular / Clumpy **G.** UV (Ultraviolet) light observation (254 nm and 365 nm): (1) Fluorescence (Light-blue) **H.** (2) Non-fluorescence . **A** and **B** were photographed by a digital camera. **C**, **D**, **E** and **F** were viewed under a stereo microscope with 2.0 × magnification. **G** and **H** were photographed under ultraviolet light.

Chemical analysis of the collected *Stereocaulon* specimens

For each sample, a total of 10 g of the lichen was soaked in acetone at room temperature for 7 days, then each solvent was filtered and concentrated under vacuum using a rotary evaporator with low pressure and temperature at 28 °C. Concentrated crude extracts were diluted and transferred to pre-weighed vials. Then the crude extracts were reconcentrated and weighed again to calculate the crude extract weight. After that, the crude extracts were desiccated and stored in a freezer at –20 °C for later processing.

Spot tests were conducted directly on the thallus of each sample by observing the colour changes under a stereo microscope (Ismed et al., 2018) after applying the different treatments/reagents in the following order: (1) Ultraviolet (UV-test), (2) Potassium hydroxide solution (K-test), (3) Sodium hypochlorite solution (C-test), (4) KC-test, (5) *para*-phenylenediamine solution (PD-test), and (6) Iodine solution (I-test).

Next, thin-layer chromatography (TLC) was conducted following the methods by Orange et al. (2001) on silica gel plates 10 cm × 20 cm (Kieselgel 60 F258, Merck, Germany). Then, a few drops of each concentrated crude extract, dissolved in acetone, were spotted at the line of origin (1 cm from the bottom) on TLC gel plates. A 10 mL volume of the solvent system Toluene:Dioxane:Acetic acid (180:45:5 v/v/v, corresponding to 7.8 mL:2.0 mL:0.2 mL respectively) was prepared and poured into a labelled developing chamber. The solvent front was allowed to migrate to 0.5 cm from the top of the plate (the ending line). TLC plates were placed into the chamber for chromatographic development.

After the TLC plate was fully developed in the chamber, the plate was taken out and air dried. The TLC plate was then observed and recorded under ultraviolet (UV) light (254 nm and 365 nm). After observation under ultraviolet light, the plate was sprayed with a molybdophosphoric acid visualisation reagent and heated on a hot plate.

For high-performance thin-layer chromatography (HPTLC), solvent system A (Toluene: Dioxane: Acetic acid, 180:45:5 v/v/v) was prepared. Approximately 2 µL of each crude extract was applied to the HPTLC plate for development. Each extract was sprayed automatically by the HPTLC system onto an HPTLC glass plate. The plate was then placed into the developing chamber. The glass plate was then observed in the visualisation chamber of the HPTLC system under 254 nm, 365 nm and white light. Retention factor (R_f) values were measured by using the CAMAG software. All results from TLC and HPTLC were compared with reference chemical data provided by Orange et al. (2001).

Genetic sequence isolation of the *Stereocaulon* lichens

A total of selected 24 *Stereocaulon* samples that represent all morphological groups from different localities were included in a genetic analysis. A specimen of *Cladonia* sp. was included as an outgroup because *Cladonia* is closely related to *Stereocaulon* (Genbank Accession no. PP158545). DNA was extracted from the thallus (around 1 cm long) by using QIAGEN Plant Mini Kit and its extraction protocols. The nuclear internal transcribed spacer (ITS rDNA; ITS1–5.8S–ITS2) was amplified with primers (1) ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and (2) ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Gardes & Bruns, 1993; White et al., 1990). PCR was performed under the following conditions: an initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturing at 94 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min. The cycles were followed by a final extension at 72 °C for 8 min. All samples were amplified in a total reaction volume of 25 µL (20 µL of Qiagen® Taq PCR Master Mix Kit and 5 µL DNA extract).

The positive PCR samples were sequenced at Apical Scientific Sdn. Bhd. All the sequences were deposited in NCBI GenBank.

The phylogenetic relationships of the selected samples were estimated by using Bayesian and Maximum Likelihood (ML) approaches (Fig. 4). Bayesian analyses were run in MrBayes v3.2.7 for 10 million iterations, with tree sampling every 100 iterations; the first 25,000 trees (20 %) were discarded as burn-in, while the rest were used to obtain the final consensus tree. The ML analyses were run in IQ-Tree v 2.2.2.7 with the TNe+I model and 1000 rapid bootstrap iterations. The genetic interspecific (i.e., inter-OTUs) and intraspecific (i.e., intra-OTU) distances were also calculated in MEGA ver. 10 (Kumar et al., 2018).

Delimitation of specimens as Operational Taxonomic Units (OTUs)

The 42 specimens were initially assigned to five operational taxonomic units (OTUs) based on morphological similarities as follows: (1) RF (ramular-fluorescence), (2) RN (ramular-nonfluorescence), (3) GF (granular-fluorescence), (4) GN (granular-nonfluorescence), and (5) RU (ramular-curved). To cross-validate these morphologically-defined OTUs, their chemical compositions were examined to assess homogeneity within each OTU and differentiation between them, and the obtained phylogeny was used to further evaluate their delimitation based on monophyly and genetic distances among these OTUs.

RESULTS

A total of 40 *Stereocaulon* specimens were collected between 1900 m and 3700 m a.s.l. along the summit trail of Mount Kinabalu. Based on our observation, the genus appeared to be most common and abundant at elevations between 2600 m and 3300 m a.s.l. The habitats in this elevation zone are dominated by *Leptospermum recurvum* forests and scrubs on ultrabasic and granite bedrock (Table 1; Fig. 1). In addition, two specimens were obtained from Mount Alab, collected at 1900 m a.s.l.. All 42 samples were found in open areas with direct sunlight exposure, directly attached to soils or rock surfaces and growing in patches.

Table 1: Details of *Stereocaulon* samples collected from Mount Kinabalu, Sabah, with collector number, BORNEENSIS Herbarium (BORH) accession number, operational taxonomic unit (OTU) number, location information, and GenBank accession number for the ITS sequences.

Collector No.	Herbarium Accession No.	*Stereocaulon* OTUs	Location	Coordinates	Elevation (m)	Genbank accession no.
JK01	BORH 5854	RU2	Mt. Kinabalu, Summit trail	N 6.03651, E 116.55039	2112	PP158542
JK02	BORH 5855	RU2	Mt. Kinabalu, Summit trail	N 6.03767, E 116.55023	2129	PP158543
JK03	BORH 5856	RU2	Mt. Kinabalu, Summit trail	N 6.03973, E 116.55036	2223	PP158544
JK05	BORH 5858	RN	Mt. Kinabalu, Summit trail	N 6.04302, E 116.55978	2648	PP158546
JK06	BORH 5859	RU2	Mt. Kinabalu, Summit trail	N 6.04421, E 116.56005	2679	-
JK09	BORH 5862	RU2	Mt. Kinabalu, Summit trail	N 6.04510, E 116.55987	2691	-
JK10	BORH 5863	RF	Mt. Kinabalu, Summit trail	N 6.04510, E 116.55987	2691	PP158547

JK11	BORH 5864	RU2	Mt. Kinabalu, Summit trail	N 6.04556, E 116.56008	2708	-
JK12	BORH 5865	RU2	Mt. Kinabalu, Summit trail	N 6.04600, E 116.56026	2724	PP158548
JK13	BORH 5866	RN	Mt. Kinabalu, Summit trail	N 6.04600, E 116.56026	2724	PP158549
JK14	BORH 5867	RU2	Mt. Kinabalu, Summit trail	N 6.04799, E 116.56295	2880	-
JK15	BORH 5868	RU1	Mt. Kinabalu, Summit trail	N 6.04975, E 116.56342	2921	-
JK16	BORH 5869	RF	Mt. Kinabalu, Summit trail	N 6.04975, E 116.56342	2921	PP158550
JK17	BORH 5870	RU1	Mt. Kinabalu, Summit trail	N 6.05137, E 116.56378	2932	-
JK18	BORH 5871	RU1	Mt. Kinabalu, Summit trail	N 6.05108, E 116.56360	2943	-
JK19	BORH 5872	RF	Mt. Kinabalu, Summit trail	N 6.05136, E 116.56381	2964	-
JK20	BORH 5873	RU2	Mt. Kinabalu, Summit trail	N 6.05163, E 116.56415	2982	-
JK21	BORH 5874	RF	Mt. Kinabalu, Summit trail	N 6.05190, E 116.56414	2994	-
JK22	BORH 5875	RF	Mt. Kinabalu, Summit trail	N 6.05328, E 116.56454	3038	-
JK23	BORH 5876	RF	Mt. Kinabalu, Summit trail	N 6.05527, E 116.56460	3118	PP158551
JK24	BORH 5877	RU1	Mt. Kinabalu, Summit trail	N 6.05589, E 116.56523	3130	-
JK25	BORH 5878	GF	Mt. Kinabalu, Summit trail	N 6.05883, E 116.56601	3239	-
JK26	BORH 5879	RU1	Mt. Kinabalu, Summit trail	N 6.05883, E 116.56601	3239	PP158552
JK27	BORH 5880	RF	Mt. Kinabalu, Summit trail	N 6.05883, E 116.56601	3239	-
JK28	BORH 5881	RF	Mt. Kinabalu, Summit trail	N 6.06548, E 116.56509	3668	PP158553
JK29	BORH 5882	RF	Mt. Kinabalu, Summit trail	N 6.06548, E 116.56509	3668	PP158554
JK30	BORH 5883	RN	Mt. Alab	N 5.82371, E 116.34117	1943	PP158555
JK31	BORH 5884	RU2	Mt. Alab	N 5.82371, E 116.34117	1920	PP158556
JK32	BORH 5885	RF	Mt. Kinabalu, Summit trail	N 6.05521, E 116.56457	3089	-
JK33	BORH 5886	RF	Mt. Kinabalu, Summit trail	N 6.05521, E 116.56457	3089	PP158557
JK34	BORH 5887	RN	Mt. Kinabalu, Summit trail	N 6.05521, E116.56457	3089	PP158558
JK35	BORH 5888	RU1	Mt. Kinabalu, Summit trail	N 6.05521, E 116.56457	3089	PP158559
JK36	BORH 5889	RF	Mt. Kinabalu, Summit trail	N 6.05527, E 116.56460	3259	-
JK37	BORH 5890	GF	Mt. Kinabalu, Summit trail	N 6.05527, E 116.56460	3259	PP158560
JK38	BORH 5891	GF	Mt. Kinabalu, Summit trail	N 6.05527, E 116.56460	3259	PP158561
JK39	BORH 5892	RF	Mt. Kinabalu, Summit trail	N 6.06548, E 116.56509	3677	-
JK40	BORH 5893	RF	Mt. Kinabalu, Summit trail	N 6.06548, E 116.56509	3677	PP158562

JK41	BORH 5894	RU1	Mt. Kinabalu, Summit trail	N 6.06548, E 116.56509	3677	PP158563
JK42	BORH 5895	GN	Mt. Kinabalu, Summit trail	N 6.06548, E 116.56509	3677	PP158564
JK43	BORH 5896	RF	Mt. Kinabalu, Summit trail	N 6.06548, E 116.56509	3677	-
JK44	BORH 5897	RN	Mt. Kinabalu, Summit trail	N 6.06548, E 116.56509	3677	PP158565
JK45	BORH 5898	GN	Mt. Kinabalu, Summit trail	N 6.06548, E 116.56509	3677	PP158566

Characterisation of the genus *Stereocaulon* morphology

All 42 *Stereocaulon* samples were of the fruticose type and belonged to five morphological groups, namely, RF (ramular-fluorescence), RN (ramular-nonfluorescence), GF (granular-fluorescence), GN (granular-nonfluorescence), and RU (ramular-curved), based on the shape of the secondary thallus (pseudopodetia), type of phyllocladia, and colour changes of the thallus under UV light. Other morphological features examined, such as cephalodia characteristics and various character measurements, did not show significant differences in the classification of *Stereocaulon* collected from Mount Kinabalu.

High-performance Thin-layer Chromatography (HPTLC)

The samples in each of the morphological groups, except for RU (ramular-curved), exhibited unique and internally homogeneous chemical profiles (e.g., shape of development, colour of bands, and R_f values of specific compounds) (Fig. 3). All samples in the RU group shared identical morphological characteristics, but these samples had two distinctive chemical profiles (hereafter RU1 and RU2). RU2 exhibited a large, smeared spot around R_f 0.40 to 0.45, and RU1 exhibited a large, smeared spot around R_f 0.50 to 0.60 under 254 nm UV observation (Fig. 3). RU1 exhibited a large, light blue coloured smeared spot around R_f 0.60, while RU2 did not exhibit any such smeared spot under 365 nm UV observation. Based on their R_f values and comparison with reference data (Orange et al., 2001; Supplementary Data 1), putative chemical compounds identified included atranorin (R_f ~ 0.90), lobaric acid (R_f ~ 0.45), and stictic acid (R_f ~ 0.47) (Fig. 3).

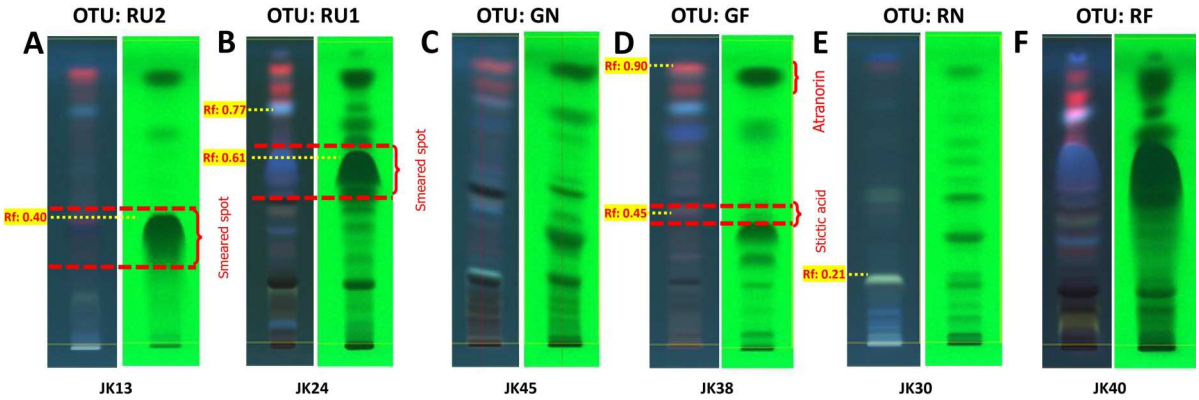

Figure 3: Comparison of chemical profiles obtained from High-performance Thin-layer Chromatography (HPTLC) of the *Stereocaulon* operational taxonomic units (OTUs) from Mount Kinabalu, Sabah: RF (ramular-fluorescence), RN (ramular-nonfluorescence), GF (granular-fluorescence), GN (granular-nonfluorescence), RU1 (ramular-curved 1) and RU2 (ramular-curved 2). The green images (right) show HPTLC profiles under UV 254 nm, and dark blue images (left) show HPTLC profiles under UV 365 nm for each *Stereocaulon* OTU. Selected distinctive chemical spots that contribute to the unique profile of each OTU, with the examples of *Stereocaulon* secondary metabolites, such as atranorin and stictic acid, are noted where identified with R_f values.

Genetic analysis and phylogenetic trees based on ITS rDNA of Mount Kinabalu *Stereocaulon* specimens

The monophyly of the six OTUs, which were determined by the morphological and chemical data, was confirmed by phylogenetic analysis with high support values (PP > 0.95; ML > 95) (Fig. 4). The sister clades of GN–GF and RU1–RU2 were phylogenetically closely related. The genetic distances among most of the OTU pairs were higher than 0.160 (16%), with a range between 0.160 and 0.222 (Table 2). The smallest genetic distances were between GN and GF (0.043) and between RU1 and RU2 (0.058). These small inter-OTU genetic distances were still much larger than the genetic distances among samples within each OTU.

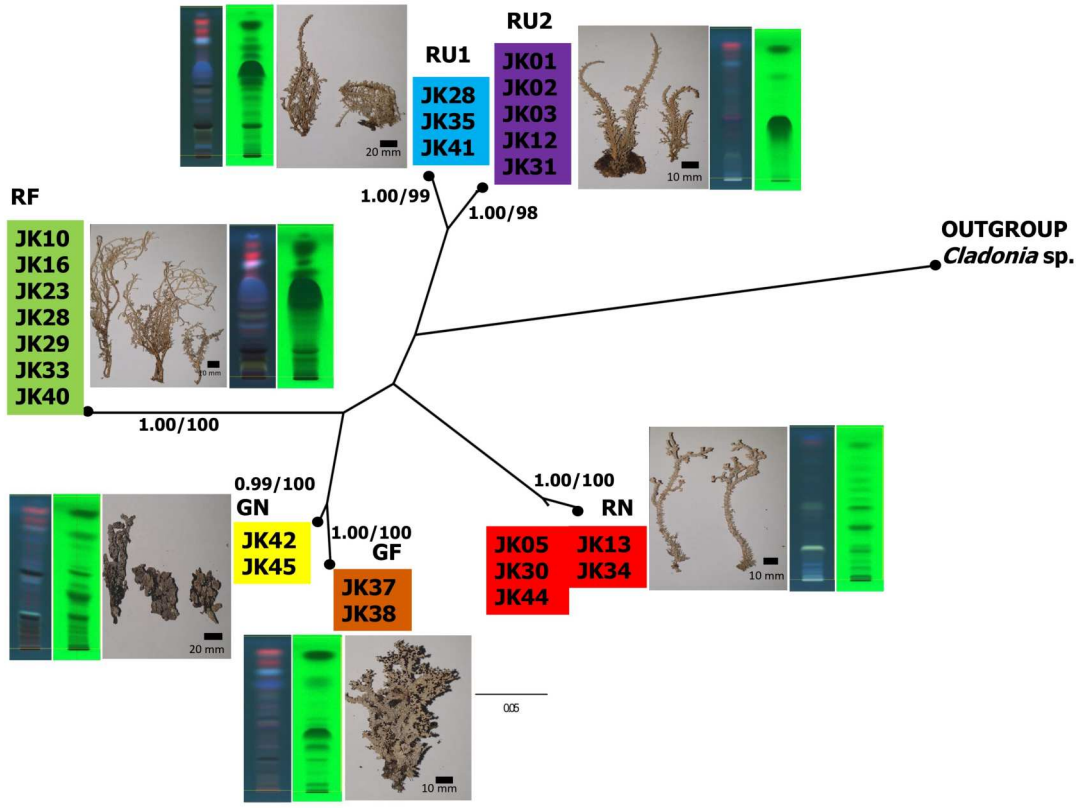

Figure 4: Phylogenetic tree (ITS rDNA) of *Stereocaulon* from Mount Kinabalu, Sabah constructed using Bayesian inference (BI) and was rooted using *Cladonia* sp. as an outgroup. Values at the nodes represent Bayesian posterior probability (left) and Maximum Likelihood bootstrap support values (right). The scale bar shows the estimated number of substitutions per site. Clades are labelled and colour-coded as follows: RN (Red), GF (Orange), GN (Yellow), RF (Green), RU1 (Blue), and RU2 (Purple).

Table 2: The average Kimura 2-Parameter (K2P) genetic distances of ITS region sequences between OTUs (inter-OTU) and within each OTU (intra-OTU; bolded values) for *Stereocaulon* from Mount Kinabalu, Sabah.

	RU2 (n = 10)	**RU1** (n = 7)	**RN** (n = 5)	**GF** (n = 3)	**GN** (n = 2)	**RF** (n = 15)
RU2	**0.001**					
RU1	0.058	**0.000**				
RN	0.196	0.202	**0.015**			
GF	0.187	0.180	0.183	**0.000**		
GN	0.169	0.160	0.175	0.043	**0.000**	
RF	0.222	0.209	0.203	0.177	0.169	**0.001**

Summary of Diagnostic Characters

The full morphological description and chemical profile information, and genetic data for each of the six OTUs, namely, RF (Ramular-Fluorescent), RN (Ramular-Nonfluorescent), GF (Granular-Fluorescent), GN (Granular-Nonfluorescent), RU1 (Ramular-Unbranching 1) and RU2 (Ramular-Unbranching 2) are given in this section.

OTU 1: RN (Ramular-Nonfluorescent) (Fig. 5)

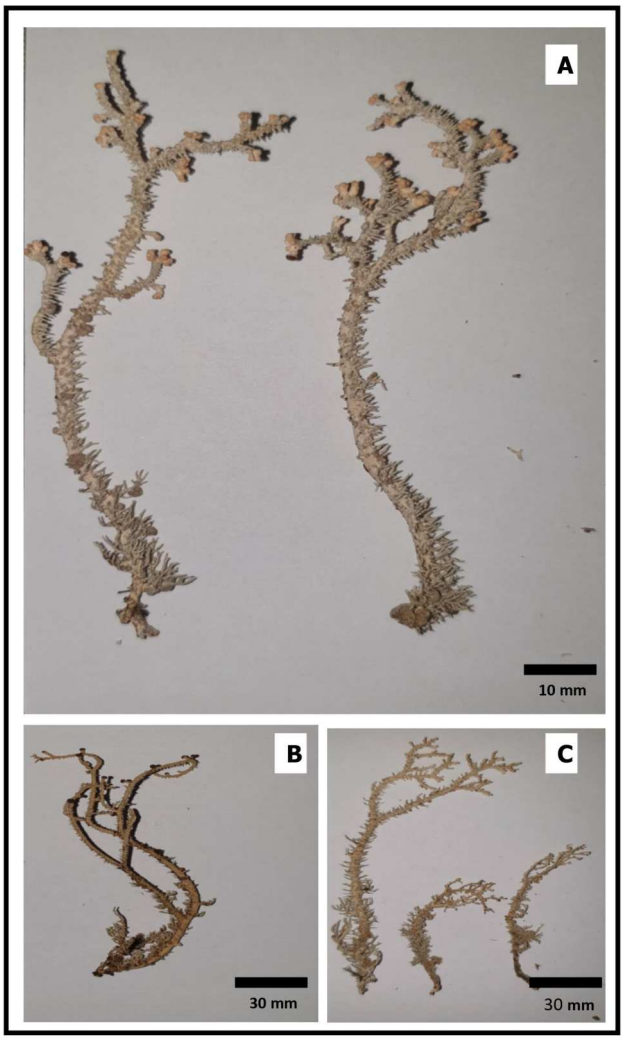

Figure 5: *Stereocaulon* specimens collected from Mount Kinabalu, Sabah that belong to the RN (Ramular-Nonfluorescent) OTU with branching secondary thallus.

Description. Pseudopodetia, 9.0–11.0 cm tall, highly branched, especially at the base and terminal parts; phyllocladia as highly branched, phyllocladioid branchlets, 0.1–5.0 mm long; cephalodia whitish-grey, wrinkled, wart-like, located randomly on pseudopodetia, 0.5–4.0 mm in diameter; apothecia 0.8–1.0 mm in diameter, dark brown, abundant terminally on pseudopodetia.

Chemistry. Atranorin and stictic acid. Spot tests: K+ yellow, PD+ yellow.

Notes. Characterised by its tall and branching pseudopodetia, phyllocladia in the form of phyllocladioid branchlets, large apothecia, and no fluorescence under UV lights (254 nm and 365 nm).

Habitat. Occurs exclusively in exposed, open sites such as on soil or rocky habitats (e.g., cliffs, rock surfaces) with no or low vegetation coverage on Mount Kinabalu and Mount Alab.

Distribution. Ranging from 2648 m to 3089 m a.s.l. on Mount Kinabalu and at 1943 m a.s.l. on Mount Alab.

Possible species as compared to previously recorded species. *Stereocaulon massartianum*: Cephalodia sacculate. Spot tests: K+ yellow or occasionally K+ red; the red reaction regularly produces the characteristic red spicular crystals of the potassium salt of norstictic acid.

OTU 2: RF (Ramular-Fluorescent) (Fig. 6)

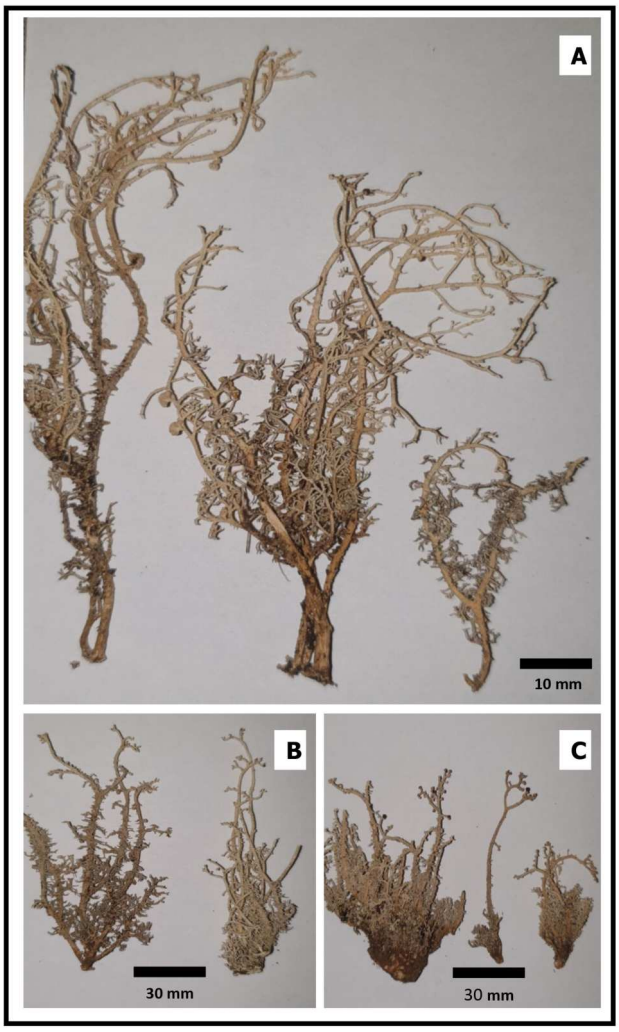

Figure 6: *Stereocaulon* specimens from Mount Kinabalu, Sabah that belong to the RF (Ramular-Fluorescent) OTU with branching secondary thallus.

Description. Pseudopodetia, 5.5–13.0 cm tall, highly branched, especially at the base and terminal parts; phyllocladia ramular, highly branched (especially at the basal areas of pseudopodetia), 1.0–8.0 mm long; cephalodia whitish-grey, wrinkled, wart-like, located randomly on pseudopodetia, 1.0–4.0 mm in diameter; apothecia 0.6–1.2 mm in diameter, dark brown, abundant terminally on pseudopodetia.

Chemistry. Atranorin, stictic acid, and lobaric acid. Spot tests: K+ yellow, PD+ yellow, UV+ white-blue fluorescence.

Notes. Characterised by its tall and branching pseudopodetia; phyllocladioid (as phyllocladia branchlets) that are highly dense at the base of the pseudopodetia; large apothecia, and white-blue fluorescence under UV light (254 nm and 365 nm).

Habitat. Occurs exclusively in exposed, open sites such as on soil, rocky habitats (e.g., cliffs, rock surfaces) with no or low vegetation coverage on Mount Kinabalu (on hiking trails and open areas).

Distribution on Mount Kinabalu. Ranging from 2691 m to 3677 m a.s.l.

Possible species as compared to previously recorded species. *Stereocaulon granulans*: Similar descriptions provided by Sipman (1993), yet with a shorter height (2–4 cm).

OTU 3: GN (Granular-Nonfluorescent) (Fig. 7)
Description. Pseudopodetia 0.5–2.5 cm tall, slightly branched terminally; phyllocladia granular and papilliform, 0.2–0.5 mm in diameter, densely aggregated terminally on pseudopodetia; cephalodia absent from the specimens that were examined in this study; apothecia 1.0–2.0 mm in diameter, dark brown to black, terminal on pseudopodetia.

Chemistry. Atranorin and stictic acid. Spot tests: K+ yellow, PD+ yellow.

Notes. Characterised by its granular and papilliform phyllocladia (abundant terminally on pseudopodetia), terminal apothecia, and no fluorescence under UV lights (254 nm and 365 nm).

Habitat. Occurs exclusively in exposed, open rocky habitats such as on rock surfaces with no vegetation coverage on Mount Kinabalu (on hornblende granite rock surface in summit zone).

Distribution on Mount Kinabalu. At 3677 m a.s.l., located in the summit zone, near the peak.

Possible species as compared to previously recorded species. *Stereocaulon graminosum*: Similar, differing only in the small size of its phyllocladia.

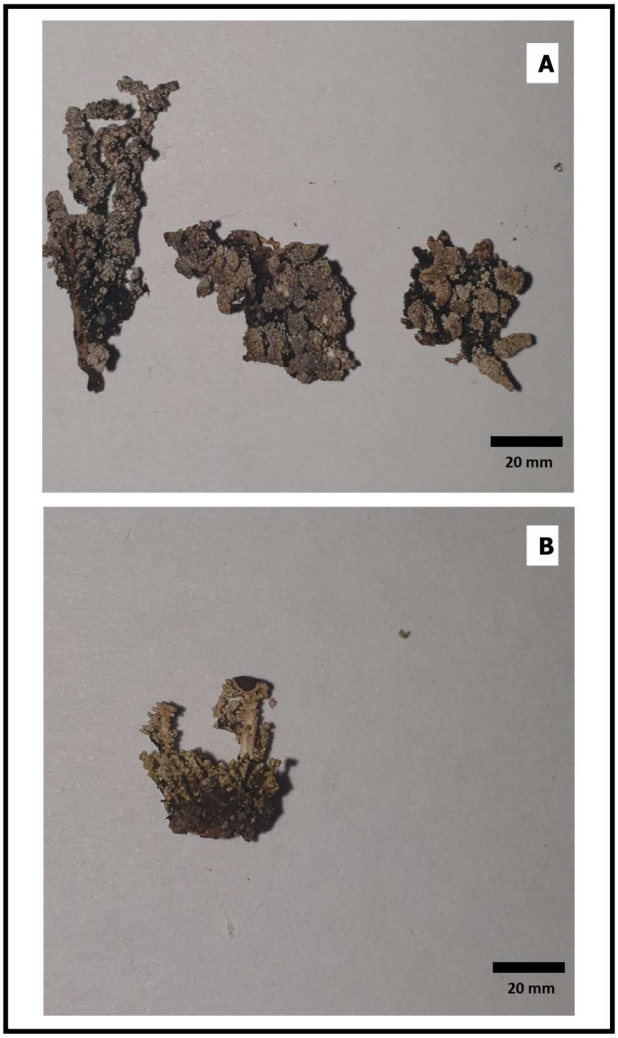

Figure 7: *Stereocaulon* specimens from Mount Kinabalu, Sabah that belong to the GN (Granular-Nonfluorescent) OTU with branching secondary thallus.

OTU 4: GF (Granular-Fluorescent) (Fig. 8)

Description. Pseudopodetia 3.0–10.0 cm tall, highly branched terminally; phyllocladia granular and papilliform, 1.0–1.5 mm long; cephalodia whitish-grey, wrinkled, wart-like, located at the basal areas of pseudopodetia, 0.8–1.5 mm in diameter; apothecia 0.5–1.0 mm in diameter, dark brown to black, highly abundant terminally on pseudopodetia.

Chemistry. Atranorin, stictic acid, and lobaric acid. Spot tests: K+ yellow, PD+ yellow, UV+ white-blue fluorescence.

Notes. Characterised by its granular and papilliform phyllocladia, large and abundant apothecia, and white-blue fluorescence under UV light (254 nm and 365 nm).

Habitat. Occurs exclusively in exposed, open sites on soil or rocky habitats (e.g., rock surfaces) with no or low vegetation coverage on Mount Kinabalu (on porphyritic granite rock surfaces in open areas).

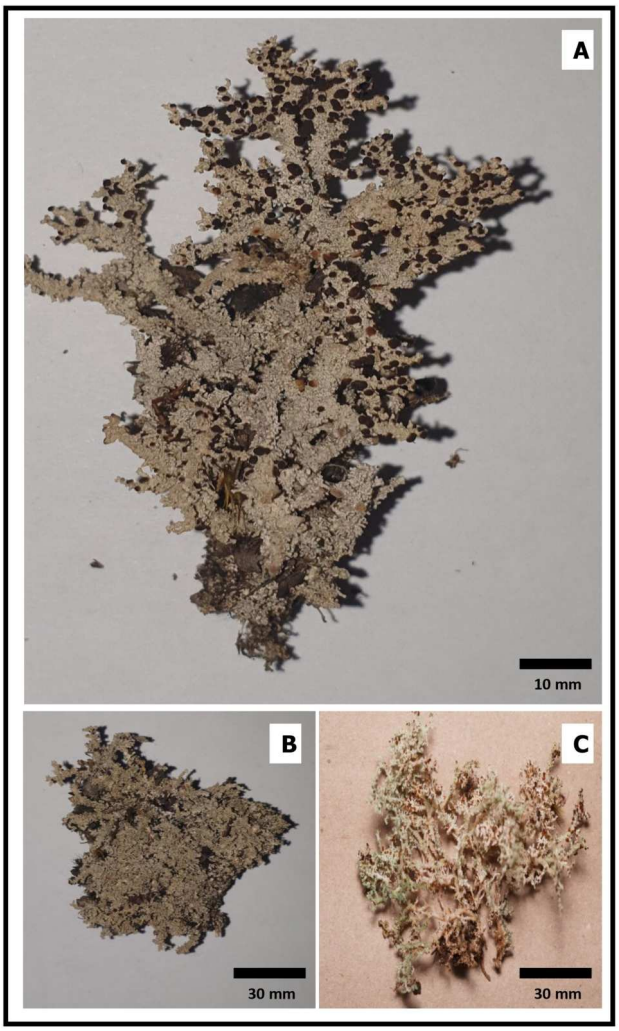

Figure 8: *Stereocaulon* specimens from Mount Kinabalu, Sabah that belong to the GF (Granular-Fluorescent) OTU with unbranching secondary thallus.

Distribution on Mount Kinabalu. Ranging between 3239 m and 3259 m a.s.l., in areas near Laban Rata.

Possible species as compared to previously recorded species. This OTU does not closely match any of the six taxa previously recorded from Mount Kinabalu by Sipman (1993). Morphologically similar species, typically found in other regions, include:

1. *Stereocaulon tomentosum*: Shorter height (2–4 cm) and larger apothecia (0.4–1.5 mm wide). Found near water sources such as lakes and river shores (Henssen, 1974; Jørgensen et al., 1998).
2. *Stereocaulon alpinum*: Shorter height (1–4 cm), and smaller cephalodia (0.3–0.8 mm diameter). Usually located in snow-covered areas; mainly circumpolar, arctic-alpine, ranging south to New Hampshire, Colorado, and Washington in North America (Lamb, 1977).

Stereocaulon grande: Slightly shorter (4–8 cm), with slightly shorter phyllocladia (0.4–0.8 mm), and slightly larger apothecia (2 mm wide). Spot tests: P– (Lamb, 1977).

OTU 5: RU 1 (Ramular-Unbranching) (Fig. 9)

Description. Pseudopodetia curved or slightly bent, 4.0–9.0 cm tall, unbranched; phyllocladia as slightly branched, phyllocladioid branchlets, 1.0–7.0 mm long, densely aggregated at the base of pseudopodetia; cephalodia whitish-grey, wrinkled, wart-like, located at the middle to basal areas of pseudopodetia, 0.5–4.0 mm in diameter; apothecia 0.1–0.8 mm in diameter, black or white, located laterally on the ends of phyllocladia, covering the middle to terminal parts of pseudopodetia.

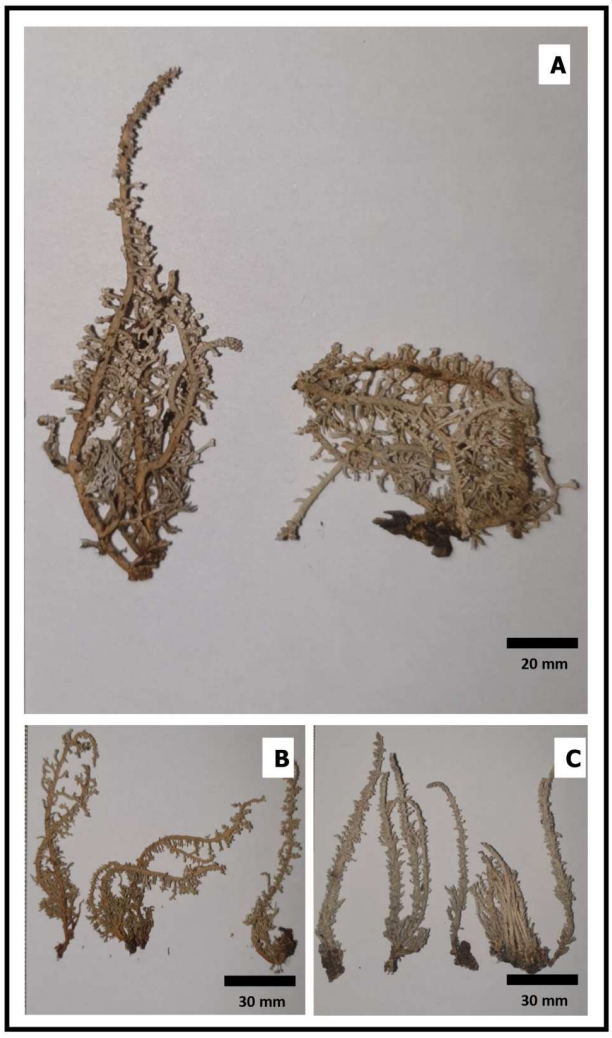

Figure 9: *Stereocaulon* specimens from Mount Kinabalu, Sabah that belong to the RU1 (Ramular-Unbranching 1) out with unbranching secondary thallus.

Chemistry. Atranorin, stictic acid, α-alectoronic acid, and lobaric acid. Spot tests: K+ yellow, KC+ red, PD+ yellow, UV+ white-blue fluorescence.

Notes. Characterised by its unbranched and curved pseudopodetia, phyllocladia as phyllocladioid branchlets; small apothecia located laterally on the end of phyllocladia, distributed from the middle to terminal areas of pseudopodetia; and white-blue fluorescence under UV light (254 nm and 365 nm).

Habitat. Occurs exclusively in exposed, open sites, on soil or rocky habitats (e.g., cliffs, rock surfaces) with no or low vegetation coverage on Mount Kinabalu (on hiking trails and open areas near Laban Rata and summit zone).

Distribution on Mount Kinabalu. Ranging from 2921 m to 3677 m a.s.l.

Possible species as compared to previously recorded species. *Stereocaulon halei*: Description similar to that provided by Lamb (1977).

OTU 6: RU 2 (Ramular-Unbranching) (Fig. 10)
Description. Pseudopodetia curved or slightly bent, 4.5–7.0 cm tall, unbranched; phyllocladia as slightly branched, phyllocladioid branchlets, 1.0–5.0 mm long; cephalodia whitish-grey, wrinkled, wart-like, located mainly at the basal areas of pseudopodetia, 1.0–3.0 mm in diameter; apothecia 0.1–1.0 mm in diameter, reddish-brown, black or white, located laterally on the end of phyllocladia, covering from the base to the terminal parts of pseudopodetia.

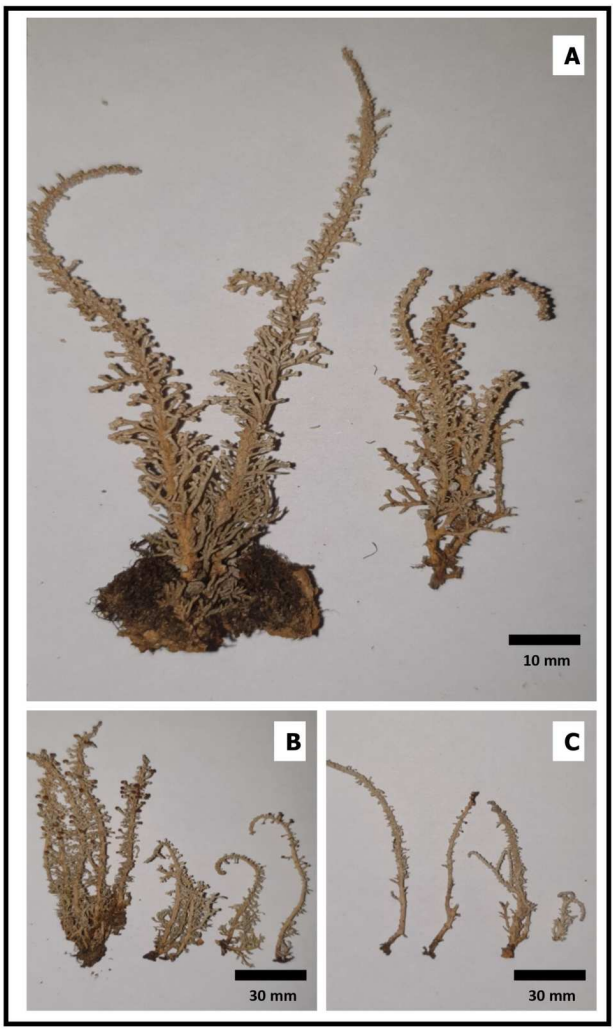

Figure 10: *Stereocaulon* specimens from Mount Kinabalu, Sabah that belong to the RU2 (Ramular-Unbranching 2) OTU with unbranching secondary thallus.

Chemistry. Atranorin, stictic acid, α-alectoronic acid, and lobaric acid. Spot tests: K+ yellow, KC+ red, PD+ yellow, UV+ white-blue fluorescence.

Notes. Characterised by its unbranched and curved pseudopodetia; phyllocladia as phyllocladioid branchlets; small apothecia located laterally on the end of phyllocladia, covering the pseudopodetia from base to terminal parts, and white-blue fluorescence under UV light (254 nm and 365 nm).

Habitat. Occurs exclusively in exposed, open sites, on soil or rocky habitats (e.g., cliffs, rock surfaces) with no or low vegetation coverage.

Distribution. Ranging from 2112 m to 2982 m a.s.l. on Mount Kinabalu and at 1920 m a.s.l. on Mount Alab.

Possible species as compared to previously recorded species. *Stereocaulon halei*: Description similar to that provided by (Lamb, 1977).

DISCUSSION

The most recent survey by Sipman (1993) recorded six *Stereocaulon* taxa from Mount Kinabalu, namely, *Stereocaulon graminosum*, *Stereocaulon granulans*, *Stereocaulon halei*, *Stereocaulon massartianum*, *Stereocaulon massartianum* var. *chlorocarpoides*, and *Stereocaulon staufferi* var. *borneense*. Although the previous species number is the same as the number of OTUs revealed in this study, we could only narrow down the possible species identities of a few OTUs. For example, RN OTU matched Sipman's morphological description of *Stereocaulon massartianum* or *Stereocaulon massartianum var. chlorocarpoides*. RF samples corresponded to *Stereocaulon granulans*, and GN OTU matched *Stereocaulon graminosum*. GF OTU similar to *Stereocaulon tomentosum*, *Stereocaulon alpinum* and *Stereocaulon grande*, despite these species are not in Sipman (1993). Both RU1 and RU2 OTUs aligned morphologically with *Stereocaulon halei*. Although only a small set of macro-morphological characters were examined in this study and successfully separated the OTUs, it is still important for future, more detailed taxonomic studies to examine other micro characters (e.g., ascus and ascospores).

Although the chemical profile data for three of the six *Stereocaulon* species from Mount Kinabalu were available, these data could not be used to make reliable species identifications because of the chemical variabilities in the same species (Sipman, 1993). Lamb (1977) reported 123 chemical compounds from *Stereocaulon* species for identification purposes, but high morphological polymorphism and chemical variability challenge the usefulness of these reference data (Ismed et al., 2018). Compared to spot tests, the use of secondary metabolite profiles data for lichen taxonomy is still underutilised (Orange et al., 2001; Huang, 2008, 2010; Oset, 2014). Our study shows that HPTLC secondary metabolite profiles are useful for the delimitation of the *Stereocaulon* specimens into OTUs and potentially into species. In our example, RU1 and RU2, which were genetically distinct but morphologically indistinguishable, could be separated by the secondary metabolite profiles. However, further research with more comprehensive datasets is necessary to verify the reliability of this method, as Huang (2008) has also noted the challenges of using chemical characters in *Stereocaulon* taxonomy.

Phylogenetic analysis supports the monophyly of the six morpho-chemically defined OTUs, suggesting distinct evolutionary lineages (Fig. 4). A critical aspect for the taxonomic interpretation in our study of these closely related sister OTU clades is that even their ITS gene genetic distances (0.043–0.058) were found to be substantially greater than the maximum intra-OTU genetic distances (0.015). This suggests a consistent genetic gap that distinguishes even these more closely related OTUs from one another (e.g. RU1–RU2; GF–GN). This interpretation aligns with findings where similar or even lower genetic distance thresholds have been applied to differentiate species in other lichen studies (Del-Prado et al. 2010, 2011; Leavitt et al., 2013; Divakar et al., 2016).

These findings underscore the power of integrating genetic sequence data, especially when attempting to resolve problematic groups that exhibit overlapping morphological and chemical profiles, including the crucial identification of potential cryptic species, as shown in *Stereocaulon alpinum* (Ekman & Tonsberg, 2002; Del-Prado et al., 2010, 2011; Leavitt et al., 2013; Fontaine et al., 2013; Divakar et al., 2016; Torres et al., 2023).

Therefore, both genetic and chemical data are valuable tools for lichenologists in making taxonomic decisions (Lamb, 1977; Lumbsch, 2002; Del-Prado et al., 2010; Ismed et al., 2018). Furthermore, it is important to include morphological, chemical and genetic data of the specimens of *Stereocaulon* species from type localities, in order to establish reliable reference data for species identification and to expand sampling efforts for each *Stereocaulon* species across multiple regions and environments to improve our understanding of the extent of morphological, chemical, and genetic variability.

CONCLUSIONS

In this study, six operational taxonomic units (OTUs) of *Stereocaulon* were identified from Mount Kinabalu, Sabah. The RN samples were identified as potentially *Stereocaulon massartianum*, the GF samples as *Stereocaulon tomentosum*, the GN samples as *Stereocaulon graminosum*, the RF samples as *Stereocaulon granulans*, and both RU1 and RU2 as *Stereocaulon halei*. Additionally, our study shows that HPTLC secondary metabolite profiles hold promise for the taxonomy of lichens. Our data provide a baseline for future studies on the taxonomy of *Stereocaulon* species in Mount Kinabalu, integrating morphology, chemistry, and genetics. Given that *Stereocaulon* species can have broad distribution ranges, further comparison of populations from different regions could shed light on the consistency of their genetic and chemical profiles that could potentially serve as diagnostic characters.

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DECLARATIONS

Research permit(s). Sabah Biodiversity Center: JKM/MBS.1000-2/2 JLD. 13(39) and Sabah Parks: TTS/IP/100-6/2 JLD.13.
Ethical approval/statement. Not applicable.
Generative AI use. The authors declare that generative AI has been used in compliance with the JTBC policies for final proofreading to correct the language error. We have reviewed and edited the content after using this tool and we take full responsibility for the content of the publication.

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APPENDIX

Appendix 1: HTPLC (High-performance thin-layer chromatography) image of all 42 samples of crude extracts of *Stereocaulon* developed using Solvent System A, under UV 254nm, 365 nm, and White light after spraying with phosphormolybdic acid.