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Corticolous crustose lichens in Pangasinan, Philippines, including one new country record

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Abstract

Corticolous crustose lichens are one of the understudied groups of lichens in the Philippines due to the limited research and insufficient taxonomical knowledge among these taxa. This study extends the inventory of corticolous crustose lichens in western Pangasinan, Philippines, specifically in Alaminos city tree park, an area established in local reforestation efforts. Morphological, anatomical and chemical characterizations were done to identify the lichen species. Character and character states of the ascomata, hymenium, exciple, ascus, ascospore and lichenic acid were examined. Moreover, a provisional dichotomous key was created. This survey identified ten lichen species across five families. The crustose families were Arthoniaceae, Coenogoniaceae, Graphidaceae, Malmideaceae and Trypetheliaceae. The species identified were *Coniocarpon cinnabarinum, Coenogonium geralense, Carbacanthographis albolirellata, Diorygma confluens, Graphis furcata, Graphis lineola, Malmidea subgranifera, Nigrovothelium inspersotropicum, Sarcographa labyrinthica* and *Trypethelium eluteriae*. The Graphidaceae family has the highest number, with four species, followed by Trypetheliaceae, with two species. The Arthoniaceae, Coenogoniaceae and Malmidaceae families have only lichen species each. Notably, *Carbacanthographis albolirellata* represents a new distribution record for the Philippines. The richness of corticolous crustose lichen species in Alaminos City Tree Park is relatively low. Nevertheless, the one newly recorded species in the Philippines added valuable insights to the limited lichen research.

Keywords

arthoniaceae; coenogoniaceae; crustose; malmideaceae; microlichens

Introduction

Lichen diversity in tropical regions remains largely unexplored due to limited research and taxonomic challenges associated with these cryptogams (1,2). Most Philippine lichens studies have concentrated on select provinces, including Benguet, Cavite, Ifugao, Ilocos Norte, Isabela, Leyte, Mountain Province, Quirino, Nueva Vizcaya, Bukidnon, Cotabato, Negros Oriental and Palawan (3-13). Compared to the 18500 species of lichens described in the world, the country has only 1264 lichen taxa with 1234 validated species names (14). Moreover, due to their cryptic morphology and identification challenges, corticolous crustose lichens are among the largest but least understood lichen groups (15). In Region 1 of the Philippines, the first account on microlichens was from the Hundred Islands National Park (HINP), Alaminos City, western Pangasinan (16). Thirty-two epiphytic lichen species were identified, spanning the families Coenogoniaceae, Graphidaceae, Lecanoraceae, Physciaceae, Pyrenulaceae, Ramalinaceae, Roccelaceae and Trypetheliaceae. Additionally, Fajardo and Bawingan identified 35 Graphidaceae microlichens species in Western Pangasinan (17). Fajardo further expanded regional research, studying manglicolous corticolous microlichens in Bangrin Marine

Protected Area, Bani, Pangasinan (18). Except for these three-lichen research studies, little is known about lichens in Pangasinan. There is a need for extensive research on this group of microlichens because of the difficulty in creating comprehensive taxonomic keys to further lichen identification and ecological studies (15).

The Department of Environment and Natural Resources (DENR) implemented the "Adopt-A-Mountain" program under Administrative Order No. 98-62, s. 1998. This strategy focuses on restoring degraded or deforested forestlands to improve vegetation cover, revive native ecosystems, enhance biodiversity and develop potential sources of water and wood. The City of Alaminos, Pangasinan, has integrated this Adopt-A-Mountain program into its environmental management approaches in its integrated coastal resource management program, which aims to develop a watershed, tree park and research area. The mountain is named Alaminos City Tree Park, located in Sabangan, Alaminos City, which is a 16.28 ha of vast forest land. It is claimed to be soon known as the City's Forest and Leisure Park (19). Although several activities and collaborations have been done in Alaminos City to re-green the mountain, little is known about the current biodiversity of organisms present-more so on pioneer organisms such as lichens and mosses. Lichens are frequently not included in ecosystem service assessments, management policies and conservation efforts, mainly due to challenges in taxonomic identification and poor understanding of their ecological importance (20). This research aims to expand knowledge of corticolous crustose lichen diversity in Pangasinan, focusing on the understudied ecosystem of Alaminos City Tree Park Forest.

Materials and Methods

Study site

The study site is Alaminos City Tree Park Forest, located in Barangay Sabangan, Alaminos City, Western Pangasinan, Northern Philippines, with the coordinates 16.130485 °N, 120.029824 °E (Fig. 1). The tree park is a tropical lowland secondary forest with native and introduced tree species such *Acacia* spp. (acacia), *Eucalyptus* spp. (eucalyptus), *Mangifera indica* (mango), *Pinus* sp. (pine), *Sandoricum koetjape (*cotton fruit) and *Swietenia mahogani* (mahogany). The collection sites were characterized by elevations ranging from 68 to 97 meters, light intensity of 54 to 9,014 lux, relative humidity of 57% to 91% and temperatures varying from 26 °C to 32 °C.

Lichen collection

The lichen survey in western Pangasinan began after the DENR issued a gratuity permit in 2019. The permit ensures that research field studies and collections are conducted responsibly, sustainably and in adherence to Philippine conservation laws and international conventions. Additionally, the Local Government of Alaminos City permitted the survey to be conducted in 2024. Only lichen thalli with necessary taxonomic features were collected with a portion of the superficial tree bark to avoid damage to the tree (21). A layer of clear nail polish was applied to collection sites on tree trunks to prevent pathogenic infections. Most samples were gathered from decaying wood and branches, with a limit of three thalli per species collected at each site for morphoanatomical and chemical studies and voucher preparation (17). The survey resulted in 95 lichen collections, now stored at the Biology Laboratory of the Natural Science Department of Pangasinan State University (PSU) - Lingayen Campus.

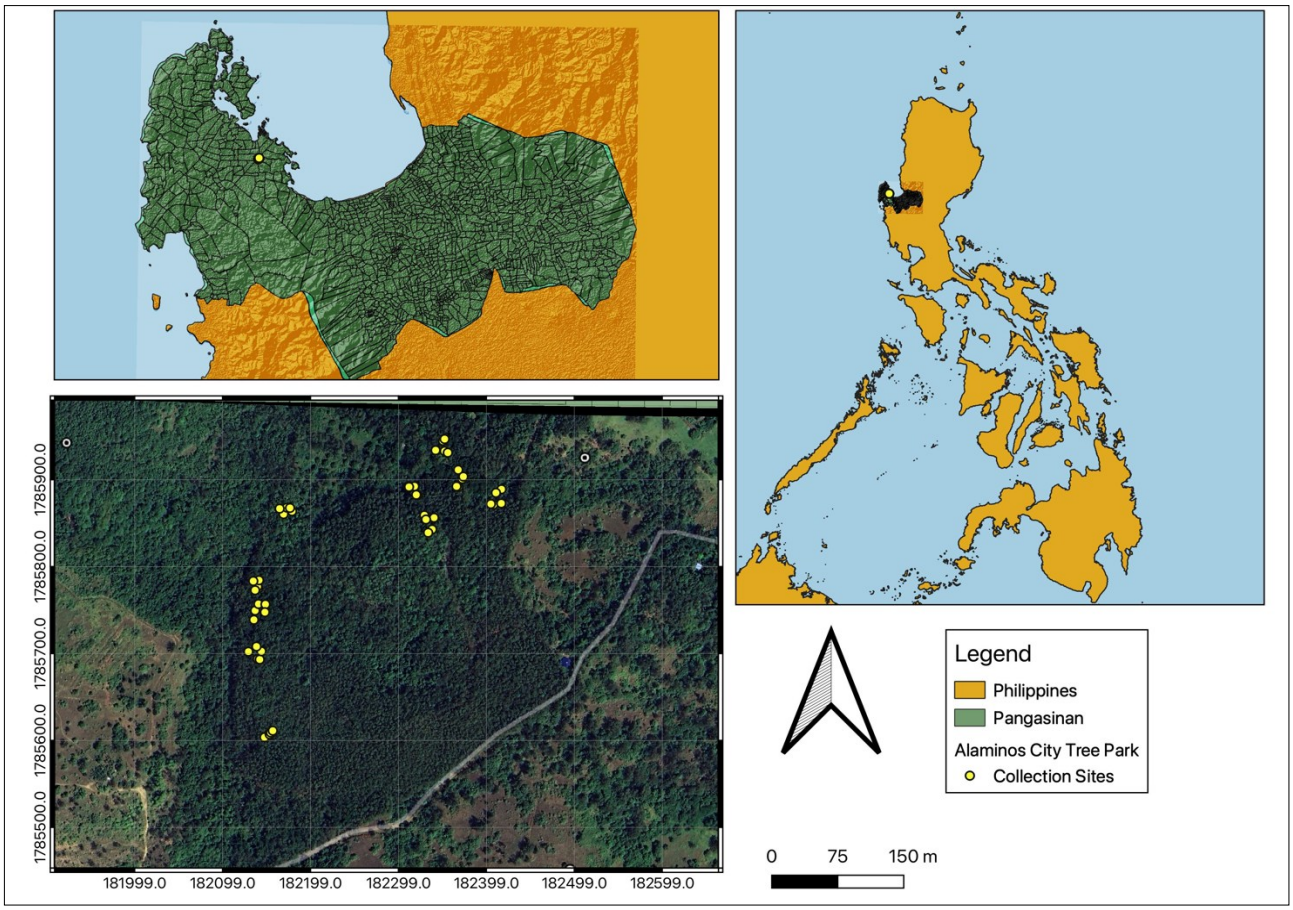

Fig. 1. Collection sites at Alaminos City Tree Park, Alaminos City, Pangasinan, Philippines.

Lichen characterization and identification

The lichen families and genera were identified based on their growth forms, reproductive structures and the presence or absence of lichen acids. Species-level identification involved microscopic examinations of the thallus, ascomata, ascus, ascospores and hymenium. Chemical tests were also conducted to detect amyloid properties and specific lichenic acids.

A stereomicroscope with a total magnification of 40x was used to examine the morphology of ascomata and thalli. For detailed morphoanatomical examination, Free-hand dissection of the ascomata, thalli and other lichen parts was done under the stereomicroscope (22). These sections were placed under a compound light microscope (Motic, Speed Fair Co., Ltd, Hong Kong) with a magnification of 400x. Morphoanatomical measurements were performed in water mounts for ascus, ascospores, crystals, epihymenium, hymenium and subhymenium (n=5). A calibrated eyepiece measured the relevant taxonomic characters (17). The detection of lichenic acid presence or absence was conducted using spot colour tests with several solutions: an iodine solution (I test), a 10% aqueous potassium hydroxide solution (K test), an aqueous solution of calcium hypochlorite (C test) and ethanolic solution of paraphenylenediamine (P test) (17). The specific lichenic acid was identified using thin-layer chromatography (TLC) following the solvent system C per the established protocol (23). The collected lichens were characterized and taxonomic keys and descriptions for *Carbacanthographis*, *Coenogonium*, *Coniocarpon*, *Diorygma*, *Graphis*, *Malmidea*, *Nigrovothelium*, *Sarcographa, Trypethelium* were used to identify the species (15,22-39).

Results and Discussion

Table 1 shows ten corticolous crustose lichen species in Alaminos City Tree Park, Barangay Sabangan, Western Pangasinan, Northern Philippines, belonging to six lichen families. The family of crustose Graphidaceae has four species, including *Carbacanthographis, Diorygma, Graphis* and *Sarcographa.* Moreover, of the ten species identified, *Carbacanthographis albolirellata* is a new distributional record (*) to the Philippines. Fig. 2. shows the morphological and anatomical features of identified lichens.

Table 1. Corticolous crustose lichen species found in Alaminos City Tree Park, Pangasinan

Family	Genus	Species
Arthoniaceae	*Coniocarpon*	*Coniocarpon cinnabarinum*
Coenogoniaceae	*Coenogonium*	*Coenogonium geralense*
	Carbacanthographis	* *Carbacanthographis albolirellata*
Graphidaceae	*Diorygma*	*Diorygma confluens*
	Graphis	*Graphis furcata*
		Graphis lineola
	Sarcographa	*Sarcographa labyrinthica*
Malmideaceae	*Malmidea*	*Malmidea subgranifera*
Trypetheliaceae	*Nigrovothelium*	*Nigrovothelium inspersotropicum*
	Trypethelium	*Trypethelium eluteriae*

Taxonomic description of Corticolous Crustose lichens in Alaminos city tree park

Genus *Carbacanthographis* Staiger & Kalb

Species in the genus *Carbacanthographis* have a prominent lirellate ascocarp that is partially to fully covered by a thalline margin. Its excipulum is either laterally or entirely carbonized. Its paraphysis tips are smooth, while the periphysoids are warty or rarely smooth. The ascospores are transversely septate to muriform, transparent and typically non-amyloid or weakly amyloid, with occasional full amyloidity and lens-shaped lumina (15,25,40-42). There are 41 known species in the recent revision of the genus (24). However, 14 additional species were discovered for the same genus as per latest study (26). The sole *Carbacanthographis* species collected from Alaminos City Tree Park, previously known only from Tamil Nadu, India, represents a new distributional record for the Philippines (25).

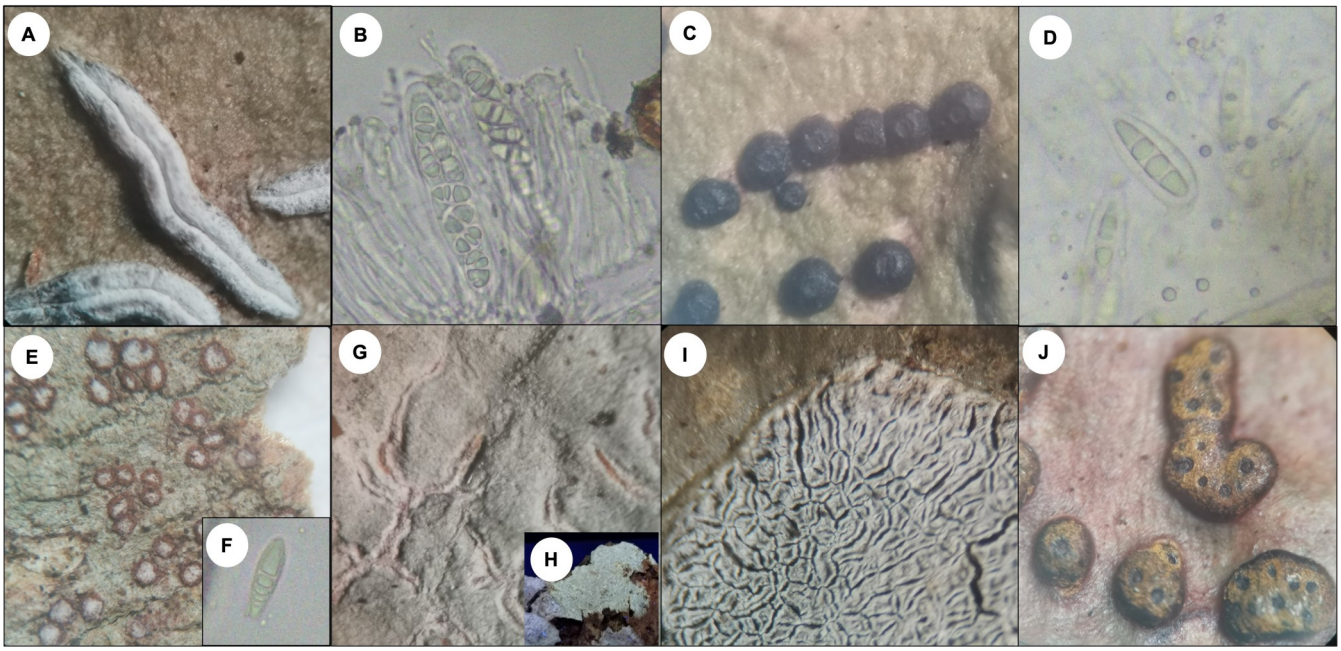

Fig. 2. A. Habitus of *C. albolirellata* (40x); B. Ascospores (15.6-18.2 × 10.4 µm) in ascus of *C. albolirellata* at (400x); C. Habitus of *N. inspersotropicum* (40x); D. Ascospores (20.8-26 × 7.8 µm) on inspersed hymenium of *N. inspersotropicum* (400x); E. Habitus of *C. cinnabarinum* (40x); F. Transversely septate macrocephalic ascospore (23.4-26 × 7.8-10.4 µm) of *C. cinnabarinum* (400x); G. Habitus of *D. confluens* (40x); H. UV+ yellow Thallus of *D. confluens* I. Habitus of *S. labyrinthica* (40x); J. Habitus of *T. eluteriae* (40x).

Carbacanthographis albolirellata B. O. Sharma & Khadilkar

Thallus is crustose, corticolous, ecorticate, greyish white, 36.4-44.6 µm thickness with thick black hypothallus. The ascocarps are lirellate, prominent to sessile, simple, flexuous with acute ends and heavily pruinose. The disc is slit-like and pruinose. Proper exciple, convergent, wholly carbonized. The epithecium is not present. The hymenium is transparent and not inspersed, non-amyloid, with a size of 73-286 × 78-83.2 µm. The ascus contains eight ascospores and periphysoids are warty. The spores are hyaline, transversely septate, 4 locular, 15.6-18.2 × 10.4 µm and non-amyloid. Chemistry tests: K-(potassium hydroxide), C-(calcium hypochlorite), Pd–(paraphenylenediamine), UV-. TLC showed no detectable lichenic acid.

Genus *Coenogonium* Ehrenberg

Coenogonium is characterized by biatorine apothecia, which are yellowish to light orange-brown. The disc of the apothecia is planar to concave with a paraplectenchymatous proper excipulum and epruinose disc. The thin asci have a narrow cylindrical shape, eight-spored, KI+ blue wall, thin apex and non-amyloid internal structures. The ellipsoid to fusiform transparent ascospores are typically one-septate, though occasionally non-septate, with unthickened walls. The wart-like or tube-like conidioma is either erumpent or superficial, containing simple or rarely septate ovoid to ovate conidium. Neither the Thallus nor the apothecia contain lichenic substances by TLC (43). The genus is relatively large, with 92 species (27,44-46). The species of *Coenogonium* found in Alaminos City Tree Park was documented to be present in several countries, including the Philippines (47).

Coenogonium geralense (Henn.) Lücking

Thallus crustose, corticolous, corticate, continuous, greyish green, 31.2-33.8 µm thickness, prothallus and isidia absent. Pycnidia are wart-shaped. Apothecia are biatorine, sessile, rounded, medium-sized, having a diameter of 0.5-0.6 mm. The disc is slightly concave to planar and the margin is smooth and thin, which is concolorous or crème-colored. Exciple has a thickness of 65-78 µm. Hypothecium is pale yellow with a thickness of 52-65 µm. The hymenium is hyaline with a thickness of 67.6-88.4 µm. Asci has eight non-amyloid ascospores. Ascospores are biseriate or irregularly biseriate, narrowly ellipsoid, one-septate, 5.2-7.8 × 2.6 µm and 3-4 times as broad. Chemistry: K-, C-, Pd-, UV-, I+ bluish hymenium .

Genus *Coniocarpon* DC.

Coniocarpon has only four recognized specie and they are present in moist tropical to warm-temperate areas worldwide (29,48,49). The taxon is identified by its rounded to lirellate ascomata with crystalline orange, red and purple quinoid pigments dissolving with a purple solution in KOH. Its ascospores are transversely septate with a macrocephalic form and at full maturity, they become brownish and exhibit granular ornamentation on the epispore (49-50).

Coniocarpon cinnabarinum DC

Thallus is crustose, corticolous, ecorticate, greyish green and continuous. A thin black prothallus is present. The ascomata are emergent, roundish to sparingly lobed, but not lirellate, with steep margins, 0.3-0.5 mm in diameter. The ascomata may be solitary or formed by 5-8 loose or dense aggregations. The disc of the ascomata has white pruina. The rim of the ascomata has the same level as the disc and has brownish orange to rusty red pruina above the white pruina. The hyaline to brownish exciple has a height of 26-28.6 µm. The brownish epithecium has a thickness of 39-41.6 µm. The hymenium has a height of 91-109.2 µm and a width of 273-286 µm. The hypothecium has a height of 26-28.6 µm. The arthonia-type ascus has a size of 54.6-59.8 × 20.8-23.4 µm; its tholus has a height of 5.2 µm. The transversely septate ascospores are hyaline to light brown when old, with a size of 23.4-26 × 7.8-10.4 µm and obovate with large apical cells. Chemistry: Proper exciple I+ blue, KI+ blue. Epithecium I+ blue, KI+ blue. Hymenium I+ reddish blue, KI+ blue. Hypothecium I+ red, KI+ blue. Tholus of the asci I+. Ascospore I. Hyaline crystals dissolve in K; Thallus and ascomata UV-. TLC: unknown acid detected.

Genus *Diorygma* Eschw.

The genus is recognized by its ecorticate thallus and lirellate ascocarp. The labia and discs of the lirellae have white pruina. Its excipulum is partially exposed, which is carbonized or faintly carbonized. It has clear hymenium with apically anastomosing paraphyses. The hyaline to rarely brownish transversely septate or muriform ascospores are I+ blue-violet or non-amyloid (15,31). Currently, the genus has 85 species (48,51), with the addition of one species discovered in China (30).

Diorygma confluens (Fee) Kalb et al.

Thallus is crustose, corticolous, ecorticate, greyish green, crystals present and soredia and isidia are absent. Ascomata are elongated, irregularly branched with acute ends, immersed to erumpent, 2-3 × 0.2-0.3 mm, with the same colour as the Thallus. The disc is exposed, has flesh colour and is thinly pruinose. The margins are entire, ecorticate and divergent. Excipulum is hyaline. The hymenium is clear with a thickness of 111.8-117 µm. Hypothecium is transparent with 91-96.2 µm thickness. Paraphyses are simple; periphysoids are absent. Ascus contains one ascospore. Ascospores are muriform, hyaline, I+ blue black, 83.2-85.8 × 26-28.6 µm, enclosed by a gelatinous sheath. Chemistry: K-, C-, Pd-, UV+. TLC: stictic acid.

Genus *Graphis* Adans.

The genus has lirellate ascomata, with well-developed labia that are convergent and often cover the disc. Its excipulum is partially to completely carbonized. The hymenium may be transparent or inspersed. Its ascospores are hyaline, transversely septate to muriform and amyloid, with a violet-blue reaction when treated with iodine (I+). Also, the Thallus is usually corticate and predominantly white-grey (15,40).

Graphis furcata Fée

Thallus is crustose, corticolous, ecorticate and greyish-green. The *caesiella* morph with exposed disc and non-striated labia. The lirella has laterally carbonized exciple with clear hymenium. The transversely septate transparent ascospores are amyloid (I+) with 18.2-26 × 5.2-7.8 µm size and 7-8 locules. Chemistry: K-, Pd-, C-. TLC: No lichenic acid detected.

Graphis lineola Ach.

Thallus is crustose, corticolous, ecorticate and greyish-green. The immersed in erumpent ascocarp has a lineola morph, with the disc not exposed and having an entire labia. The lirella has laterally carbonized exciple with inspersed hymenium. The transversely septate transparent ascospores are amyloid (I+) with 20.8-31.2 × 5.2-7.8 µm size and 6-8 locules. Chemistry: K-yellow, Pd-, C- TLC: no lichenic acid detected.

Genus *Malmidea* Kalb, Rivas Plata & Lumbsch

The genus is recognized by the presence of goniocysts in the Thallus, sessile, rounded biatorine apothecia, which are covered with aquaphobic granules and a hymenium that is hyaline and turns blue with iodine (I+). The ascus has simple ascospores and lacks a tubular structure (52).

Malmidea subgranifera (Kalb & Elix) Kalb & Elix

Thallus is crustose, corticolous, greyish green with goniocysts (100-200 µm). Prothallus is absent. Warts are hemispherical, not developing into soralia. Algae green about 7.6-9.2 µm. The apothecia are sessile with a 0.5 -0.7 mm diameter. The disc is brown, epruinose and planar to concave. The thinner inner margin of apothecia is greyish to black; the thicker outer margin is cream to concolourous with the Thallus and has goniocysts. The hymenium is hyaline with a height of 65-83.2 × 343.2-390 µm. The epithecium is light with 13-15.6 µm height. The hypothecium is hyaline to light brown with a 127.4-135.2 µm thickness. The black granifera-type excipulum has a thickness of 65-70.2 µm. Asci are amyloid and cylindrical with a size of 36.4-44.2 × 13-15.6 µm. Each ascus has eight simple ellipsoidal transparent ascospores. The ascospores are 10.4-13 × 5.2 µm, with evenly thickened walls. Pycnidia are not observed. Chemistry: Thallus and apothecia are UV-, Thallus K+ yellow, C- and Pd-. TLC: No lichenic acid detected.

Genus *Nigrovothelium* Lücking, Nelsen & Aptroot

The genus is distinguished by its olive-green to brown corticate Thallus. The prominent, sessile and black perithecia have apical ostioles, typically solitary but often found in dense clusters. The hamathecium is clear and hyaline, composed of thin filaments and anastomosing paraphysoids. The clavate asci contain hyaline, transversely 3-septate astrothelioid ascospores with diamond-shaped lumina (53). The *Nigrovothelium* found in Alaminos City Tree Park was discovered in the Republic of Seychelles and is also present in countries Australia, Brazil, China, Colombia, El Salvador, Guyana, Indonesia, Mexico, Papua New Guinea, Puerto Rico, Singapore and Thailand (37,47).

Nigrovothelium inspersotropicum Aptroot & Diederich

Thallus is crustose, corticolous, continuous, corticate, greenish. A thick black prothallus is present. The apothecia are black, solitary, densely crowded, prominent to sessile and 0.4-0.5 mm in diameter. The apothecia have apical ostioles and are flattened to concave slightly, surrounded by a dark brown ring. The hamaethecium is inspersed with a round to elongate oil droplets with a diameter of 2.6 µm. Each ascus has eight hyaline 3-septate ascospores. Each ellipsoid ascospore has rounded ends, angular lumina, thickened endospores and a size of 20.8-26 × 7.8 µm. Pycnidia were not observed. Chemistry: Thallus and apothecia are UV-, K- yellow, C- and Pd-. TLC: No lichenic detected.

Genus *Sarcographa* Fée

The genus is recognized by its smooth, glossy Thallus lacking isidia and soredia, a white to pale olive-green colour. It has simple to highly branched lirellate ascomata, rounded to oval. The lirellae with open discs are immersed within prominent stromata. The exciple may be either non-carbonized, basally carbonized, or occasionally wholly carbonized. The hymenium is inspersed and does not react with iodine (I-). The pale brown transversely septate ascospores have 4-10 non-amyloid lenticular locules (54).

Sarcographa labyrinthica (Ach.) Müll. Arg.

Thallus is crustose, corticolous, continuous, ecorticate and greyish green. Black prothallus is present. The apothecia are lirellate, immersed with raised white stroma. The stromatoid apothecia are rounded, oval, or distorted ellipsoid with a diameter of 3.5 mm-4.7 mm. The disc of the lirellae is matte black and thinly pruinose. The exciple is completely carbonized. The inspersed hymenium has a height of 67.6-78 µm. The globules present in the hymenium are less than 2.6 µm. Each ascus has eight light-brown ascospores. Each transversely septate ellipsoid ascospore has four locules with 13-15.6 × 5.2-7.8 µm. Chemistry: Thallus and apothecia are UV-, K+ yellow, C- and Pd-. TLC: stictic acid.

Genus *Trypethelium* Spreng.

The genus is identified by a corticate thallus that can be green, grey, yellow, or brown, with a smooth to irregular texture, sometimes slightly bullate. The prominent or sessile pseudostroma covers the black and rounded perithecia or occasionally breaks through the Thallus, often with the sides covered by the Thallus. The hamathecium is hyaline or contains oil droplets. Each ascus contains eight ascospores, which are colourless, fusiform with slightly pointed ends and transversely septate without constriction at the septa. A gelatinous sheath covers young ascospores and the lumina becomes rectangular as they mature. Conidiomata are rarely present. Lichexanthone or anthraquinones are usually present in Thallus and/or pseudostromata (36).

Trypethelium eluteriae Spreng.

Thallus is crustose, corticolous, continuous, corticate, greenish. Prothallus is present. The ascomata are trypethelioid with pseudostroma. The prominent to sessile black perithecia are 1-2 mm in diameter and are covered by yellow pigment except on the ostiole. The hamathecium is clear. Each ascus has eight hyaline ascospores. Each transversely septate fusiform ascospore has 8-12 locules with 39-44 × 7.8 µm. Chemistry: Pseudostroma is UV+ reddish orange anthraquinone, K+ reddish/purplish. Thallus UV-, K-, C- and Pd-.

Key to the species of corticolous microlichens of Alaminos City Tree Park

1a Ascomata roundish to lirellae: 2

1b Ascomata perithecia or Biatorine apothecia: 8

2a Ascomata in pseudostroma: (*Sarcographa labyrinthica*)

2b Ascomata not in pseudostroma: 3

3a Ascomata with crystalline orange or reddish pigments : (*Coniocarpon cinnabarinum*)

3b Ascomata not lined with orange or reddish pigments: 4

4a Excipulum carbonized: 5

4b Excipulum not carbonized: (*Diorygma confluens*)

5a Ascospore I^+: 6

5b Ascospore I^- :(*Carbacanthographis albolirellata*)

6a Lirellae *caesiella*-morph, hymenium clear: (*Graphis furcata*)

6b Lirellae *lineola*-morph, hymenium inspersed:(*Graphis lineola*)

7a Thallus with goniocyst :(*Malmidea subgranifera*)

7b Thallus without goniocyst: 8

8a Ascomata with yellowish to orange or light orange-brown and

biatorine apothecia: (*Coenogonium geralense*)

8b Ascomata roundish perithecia: 9

9a Ascomata are black, prominent and solitary but often found in dense clusters. (*Nigrovothelium inspersotropicum*)

9b Ascomata are black, globose and immersed in prominent to sessile yellow pseudostromata: (*Trypethelium eluteriae*)

Conclusion

The richness of corticolous crustose lichen species in Alaminos City Tree Park, Pangasinan, is relatively low. Nevertheless, the study documented 10 species belonging to six families and nine genera. This one species is newly recorded in the Philippines, adding valuable insights to the country's limited lichen research. It is recommended that a lichen study guide on corticolous crustose lichens found in Pangasinan be prepared to encourage other researchers to conduct scientific investigations on these understudied and underappreciated groups of cryptogams.

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Authors' contributions

WF was responsible for preparing the proposal, most of the morphoanatomical and chemical characterization, identifying crustose lichen species, writing results and writing the manuscript for publication. MD was involved in collecting specimens, preparing voucher specimens, organizing the references and writing the methodology part of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used CHATGPT to improve the grammar and readability of the three sentences in the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

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