

## Biological and environmental profiling of three Philippine *Tetrastigma* species (Vitaceae) in northeastern Cagayan, Luzon, Philippines

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**Abstract.** Opeña JM, Maramag CL, Alicay CB, Baloloy MV, Gaspar CA, Jr Pacris FA, Torres JS, Sumer LB, Bayani GU, Sotto RC. 2021. Biological and environmental profiling of three Philippine *Tetrastigma* species (Vitaceae) in northeastern Cagayan, Luzon, Philippines. *Biodiversitas* 22: 2956-2970. *Tetrastigma* species have considerable attention because of their medicinal importance, but little is known about these Philippine species. This study provides information on the biological (morphological, phytochemicals, nutritional, antimicrobial characterization) and environmental (diversity, distribution, growing environment, soil characteristics) profiles of the three Philippine *Tetrastigma* species namely, *Tetrastigma ellipticum* Merr., *Tetrastigma harmandii* Planch., and *Tetrastigma loheri* Gagnep. *Tetrastigma harmandii* growing in various habitats were widely distributed throughout northeastern Cagayan, while *T. ellipticum* and *T. loheri* were found only in the mountain forests of the northernmost part of northeastern Cagayan. *Tetrastigma ellipticum* was found only in Mt. Cagua forests and volcano, while *T. loheri* was found growing in the forests of Palaui Island, Buwacag mountain, and Mt. Cagua. Most of the plant species growing within the *Tetrastigma* environments were native and endemic trees. Mountain species of *Tetrastigma* (*T. ellipticum* and *T. loheri*) grew in extremely acidic soils with high organic matter content. More secondary metabolites were detected in *T. ellipticum*, which was the only species among the three *Tetrastigma* species to contain alkaloids, flavonoids, and anthocyanin. Saponins were detected only in *T. harmandii*. Steroids were not detected in the three species. Moreover, *T. harmandii* was the only species to have an antibacterial property against *Staphylococcus aureus* and a slight inhibiting activity against *Klebsiella aerogenes*. Between the two edible *Tetrastigma* species, *T. harmandii* contains more macronutrients and micronutrients, while *T. loheri* contains higher crude protein, crude fat, and crude fiber.

**Keywords:** Chemical, antimicrobial, distribution, habitats, *Tetrastigma ellipticum*, *Tetrastigma harmandii*, *Tetrastigma loheri*

### INTRODUCTION

*Tetrastigma* (Miq.) Planch is one of the most species-rich genera of the economically and agronomically important Vitaceae family (Habib et al. 2017). The genus is characterized by its unbranched to digitately branched tendrils, dioecious sexual system, and 4-lobed stigmas in female flowers (Chen et al. 2011). *Tetrastigma* is also famous in Southeast Asia for being the host plant of the *Rafflesia* (Wen 2007; Lianah et al. 2015) where *Tetrastigma harmandii* was reported as the single host plant of *Rafflesia lagascae* (as *R. manillana*) on Mt. Makiling, Laguna, Philippines (Yahya et al. 2010). *Tetrastigma* species grow suitably in hillsides and valleys of shady and moist primary rainforests (Lianah et al. 2015). In the Philippines, there are about 24 species of *Tetrastigma*, of which 15 species are endemic. Species of the genera exhibited divergences with other Asian species at various times (Wen et al. 2013). Diversity, morphological characteristics, and geographical distribution of *Tetrastigma* species in the Philippines were reported by Wen et al. (2013) and Pelsner et al. (2016).

Recently, other *Tetrastigma* species have been subjected to pharmacological and phytochemical studies. Modern pharmacological studies showed that *T. hemsleyanum* had a wide range of pharmacological functions such as antiproliferative, antitumor, antiviral, anti-inflammatory, antidiabetic, liver protective, and immunoregulation activities (Zhong, et al. 2006; Xu et al. 2008; Yang and Wu, 2009; Ma et al. 2012; Wang et al. 2018; Ding et al. 2019; Ru et al. 2019). *Tetrastigma glabratum* plant extract was found to contain steroids, terpenoids, flavonoids, saponins, and tannins (Lianah et al. 2015), while leaves of *T. leucostaphylum* contained alkaloids, phytosteroids, saponin, diterpenes, cardiac glycosides, carbohydrate, fixed oils, and fats (Adarsh et al. 2013). Common Philippine *Tetrastigma* species specifically the *T. harmandii* is used as a souring ingredient for fish (Maghirang et al. 2018) and was found to have anti-scabies, diuretic properties (Brown, 1920) and could treat urinary diseases (Quisumbing, 1951; Carag and Buot, 2017).

However, underutilized Philippine plant species such as the *Tetrastigma* have been given low priority in most research and development programs. Little is known about

their utilization, geographical extent, morphological variation, and potential in terms of medicine and food. Also, *Tetrastigma* plants are not commonly known by the local people due to limited information. Thus it is important to document the distribution and diversity of *Tetrastigma* as well as the morphological, phytochemical, medicinal, and nutritional properties of *Tetrastigma* species found in the country.

To contribute to the database about the *Tetrastigma* species in the Philippines, exploratory trips had to be conducted to determine the existence and distribution of *Tetrastigma* species in various municipalities of northeastern Cagayan, Philippines. Additionally, it hopes to look into the *Tetrastigma* species growing in protected areas of northeastern Cagayan such as the Palau Island Protected Landscape and Seascape (an island in the northeastern extremity of Luzon) in Santa Ana, Cagayan and Mt. Cagua (an active volcano lying in the eastern border of the municipality of Gonzaga, Cagayan which is part of the Sierra Madre range in the eastern coast of Luzon). Documentation of the morphology and growing environment to include the soil characteristics and the plants that grow within the *Tetrastigma* environment need to be carried out. This study also aimed to investigate the phytochemical and antimicrobial properties of the three Philippine *Tetrastigma* species. Furthermore, it sought to determine the nutritional content of the two common edible species, *T. harmandii*, and *T. loheri*. Hence, this study will be a great help to prepare strategies for conservation, propagation, and eventually in the commercialization of these underutilized *Tetrastigma* species.

## MATERIALS AND METHODS

### Prior Informed Consent (PIC) and selection of local researchers

To satisfy the legal requirements of EO 247 (Bioprospecting) and RA 9147 (Wildlife Resources Conservation and Protection Act), prior informed consent from the Local Government Units (LGUs) through the Office of the Municipal Mayor, Municipal Environment and Natural Resources Office (MENRO) and the community/barangay was obtained through letters asking for permission to conduct the research and collect plant and soil samples in the identified areas. Moreover, a gratuitous permit was secured from the Department of Environment and Natural Resources Region 2 Office for conducting the study in Mt. Cagua and Palau Island Protected Landscape and Seascape. Selection of local researchers (forest guides, MENRO and Barangay officials, Coast Guards) was made with the stakeholders in different municipalities of northeastern Cagayan based on their indigenous knowledge of the *Tetrastigma* species resources in the study sites. The local researchers per municipality were involved during the fieldwork in their respective areas.

### Sampling site description

The municipalities in northeastern Cagayan, Philippines namely Lal-lo, Camalaniugan, Aparri, Buguey, Santa

Teresita, Gonzaga, and Santa Ana were visited for the presence of *Tetrastigma* species. Northeastern Cagayan experienced a dry season from March to July, while the wet season occurs from September to January. We assessed habitats and environments where the *Tetrastigma* species grew. The presence of *Tetrastigma* species was investigated in various habitats (sole or combination). The latitude, longitude, and elevations were recorded using the Geocam Pro version 5.34 (Wazar-Apps, 6 Place Jacques Bonsergent 75010 Paris, France) and a geographical map was generated using Google Earth Pro.

### Sampling methods

The study encountered 49 accessions (in population) of *Tetrastigma* species in 7 municipalities, namely Lal-lo, Aparri, Camalaniugan, Buguey, Santa Teresita, Gonzaga, and Santa Ana. About 5 accessions of *T. ellipticum*, 36 accessions of *T. harmandii*, and 8 accessions of *T. loheri* were collected. Accession number coding follows the year of collection and collection number. The accessions were planted in the Cagayan State University-Gonzaga *Tetrastigma* field gene bank and nursery. The species were located in different barangays of the municipalities (Table 1). A combination of walk/vehicular ride, visual encounter, and photo documentation was carried out from June to October 2020.

### Identification and morphological description of the *Tetrastigma* species

Morphological identification of the *Tetrastigma* species by referring to the species description was further performed according to Pelser et al. (2016). During the sampling, the following morphological characters were recorded: old stem shape and appearance, leaf characteristics such as leaf organization, young leaf color, number of leaflets, leaflet shape, leaf margin, type of venation on the adaxial surface, presence of leaf and petiole indumentum, and tendril morphology (Table 2).

### Multivariate analysis of the *Tetrastigma* species

Visualization of the morphological similarities/differences among the *Tetrastigma* species and their populations in northeastern Cagayan was done through Principal Coordinate Analysis (PCoA) based on Euclidean distance and Bray-Curtis similarity index. Principal Coordinate Analysis (PCoA) was done using the Paleontological Statistics Software (PAST 4.03) (Hammer et al. 2001).

### Environmental analysis

The plant species growing within the *Tetrastigma* species-environment were identified. The scientific name, English name, and common/local name were provided. Characteristics of soils where the *Tetrastigma* species grew were also determined. Soil samples where the *Tetrastigma* species grew were collected in various experimental sites. Topsoils were gathered, pulverized, subjected to air-drying at room temperature until total dryness, and were analyzed according to the method outlined by Motsara and Roy (2008). The soil pH, organic carbon (OM), available phosphorus,

available potassium, and trace elements such as copper, zinc, iron, manganese were tested through the Potentiometric method, Walkey and Black Spectrophotometric, Olsen's method, Cold Sulfuric Extraction, and DTPA extraction,

respectively. Interpretation of soil test results was guided by the rating scales of Horneck et al. (2011) for total N, total P, and total K, while the rating scales of Motsara and Roy (2008) were used for the pH and micronutrients.

**Table 1.** List of characterized *Tetrastigma* accessions and their place of collection

Accession number	Species	Collection site	Latitude	Longitude	Elevation (m asl.)	Population-specific characters
20-001	<i>T. harmandii</i> Planch.	Aparri, Cagayan	18.3797°N	121.575°E	42.06	
20-002			18.3803°N	121.58102°E	45.11	
20-003			18.3801°N	121.579°E	18.90	
20-004			18.3807°N	121.581°E	28.04	
20-006	<i>T. harmandii</i> Planch.	Camalaniugan, Cagayan	18.2646°N	121.6613°E	35.97	
20-007			18.2644°N	121.6611°E	41.15	
20-008	<i>T. harmandii</i> Planch.	Buguey, Cagayan	18.19613°N	121.83661°E	53.95	
20-009			18.19648°N	121.83664°E	49.07	
20-010			18.18765°N	121.84537°E	50.90	
20-011			18.27522°N	121.86894°E	46.94	
20-012	<i>T. harmandii</i> Planch.	Sta. Teresita, Cagayan	18.2299°N	121.88542°E	43.89	Light green young and mature leaves ( <i>T. harmandii</i> )
20-014			18.22839°N	121.8835°E	52.12	
20-015			18.21513°N	121.87775°E	52.12	
20-016			18.21561°N	121.879°E	49.99	
20-017			18.20853°N	121.90755°E	56.08	
20-018			18.23117°N	121.9505°E	74.07	
20-019	<i>T. harmandii</i> Planch.	Gonzaga, Cagayan	18.29163°N	122.1074°E	181.97	Light green mature leaves with conspicuous yellow midrib (20-020 <i>T. harmandii</i> )
20-020			18.28507°N	122.1114°E	152.10	
20-021			18.28415°N	122.11287°E	146.91	
20-022			18.28517°N	122.11428°E	134.11	
20-023	<i>T. ellipticum</i> Merr.	Mt. Cagua, Gonzaga, Cagayan	18.21905°N	122.11102°E	828.14	Dark green mature leaves with visible yellow spots (20-027 <i>T. ellipticum</i> )
20-024	<i>T. ellipticum</i> Merr.		18.21688°N	122.11425°E	862.89	
20-025	<i>T. ellipticum</i> Merr.	<i>T. loheri</i> Gagnep.	18.21934°N	122.11172°E	818.98	Red newly emerged leaves; light green young fully opened leaves ( <i>T. loheri</i> )
20-026	<i>T. loheri</i> Gagnep.		18.2254°N	122.10846°E	937.87	
20-027	<i>T. ellipticum</i> Merr.		18.22379°N	122.1033°E	861.97	
20-028	<i>T. ellipticum</i> Merr.		18.22507°N	122.10024°E	793.09	
20-029	<i>T. loheri</i> Gagnep.		18.22634°N	122.09602°E	698.91	
20-030	<i>T. loheri</i> Gagnep.	Buwacag mountains, Sta. Ana, Cagayan	18.39578°N	122.23983°E	152.10	Light green newly emerged leaves; light green young fully opened leaves ( <i>T. loheri</i> )
20-031	.		18.39592°N	122.23999°E	123.14	
20-032			18.39608°N	122.24022°E	156.06	
20-033			18.39538°N	122.23931°E	127.10	
20-034	<i>T. harmandii</i> Planch.	Palau Island, Sta. Ana, Cagayan	18.54369°N	122.15144°E	45.11	Red to reddish-yellow newly emerged leaves; red to reddish light green young fully opened leaves ( <i>T. loheri</i> )
20-036	<i>T. loheri</i> Gagnep.		18.55114°N	122.1505°E	86.87	
20-037	<i>T. loheri</i> Gagnep.		18.55207°N	122.14882°E	56.08	
20-038	<i>T. harmandii</i> Planch.	Lal-lo, Cagayan	18.13551°N	121.6983°E	100.89	Undulate, thick, large leaves; prominent leaf venation and lenticels in the old stem (20-042 <i>T. harmandii</i> )
20-039			18.13625°N	121.70089°E	107.59	
20-040			18.13709°N	121.70284°E	89.00	
20-042			18.13597°N	121.70593°E	85.95	
20-043	<i>T. harmandii</i> Planch.	Gonzaga, Cagayan	18.24956°N	121.99788°E	64.92	Large rounded old stems (20-043, 20-049 <i>T. harmandii</i> )
20-044			18.25316°N	121.99811°E	66.14	
20-045			18.25275°N	122.00239°E	70.10	
20-046			18.24962°N	122.01225°E	109.12	
20-047			18.24288°N	122.04192°E	187.154	
20-048			18.28612°N	122.01881°E	46.94	
20-049			18.34764°N	122.08643°E	39.93	
20-050			18.35026°N	122.08727°E	63.09	
20-051	<i>T. harmandii</i> Planch.	Sta. Ana, Cagayan	18.37334°N	122.12352°E	35.05	
20-052			18.41915°N	122.12968°E	32.92	
20-053			18.42862°N	122.12618°E	52.12	

**Table 2.** Morphological characters of the *Tetrastigma* species that were measured qualitatively

Character	State	Reference
Old stem shape and characteristics	(1) rounded woody/semi-woody (2) flat woody/semi-woody (3) rounded herbaceous (4) flat herbaceous	Habib et al. 2018
Leaf organization	(1) pinnately trifoliolate (2) pedately compound (3) palmately compound	Pelser et al. 2016; Habib et al. 2018
Leaflet number of mature leaves	(1) 3 leaflets (2) 4 leaflets (3) 5 leaflets (4) 6 leaflets (5) 7 leaflets	
Newly emerged leaf color	(1) light green (2) red (3) reddish yellow	
Leaflet shape	(1) elliptical (2) elliptic-oblong (3) lanceolate	
Leaf margin	(1) serrate (2) entire (3) crenate	Habib et al. 2018
Type of venation	(1) cross-venulate (2) pinnate	
Leaf and petiole indumentum	(1) absent (2) present in the leaf, absent in the petiole (3) absent in the leaf, present in the petiole (4) present in both leaf and petiole	Habib et al. 2018
Tendril morphology	(1) forked/branched (2) unforked/unbranched	Pelser et al. 2016; Habib et al. 2018

### Biochemical and microbial screening in the *Tetrastigma* species

#### Preparation of the ethanolic leaf extracts

Freshly collected young and mature leaves of the *Tetrastigma* species were washed thoroughly with running tap water and rinsed thrice with distilled water. The leaves were air-dried at room temperature for 3-4 days and then chopped into smaller pieces. The air-dried leaves were oven-dried at 50°C for several hours, ground and pulverized using mortar and pestle, and sieved. The fine powder was then mixed with 90% ethanol (200 g l<sup>-1</sup>) at room temperature and filtered. The crude extracts were collected for phytochemical and microbial analyses.

#### Phytochemical screening

The leaf crude extracts were subjected to screening for the presence of the eight secondary metabolites, namely alkaloids, flavonoids, phenols, terpenoids, anthocyanin, tannins, steroids, and saponins. These were determined following the procedures of Guevarra (2005). Phytochemical screening of alkaloids, anthocyanin, flavonoids, phenols, saponins, steroids, tannins, and terpenoids was done through Meyer's test, NaOH test, Shinoda test, Ferric Chloride test, Froth test, Liebermann-

Burchard reaction, Lead Acetate test, and Salkowski test, respectively.

#### Microbial sensitivity test

The ethanolic leaf extracts were subjected to antimicrobial susceptibility test against gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Klebsiella aerogenes*, and *Acinetobacter baumannii*; and gram-positive bacteria, namely *Staphylococcus aureus* and *Enterococcus faecium* using the paper disc diffusion method (Guevarra, 2005). Bacterial strains were obtained from the Central Analytical Laboratory of the Cagayan State University, Tuguegarao City, Cagayan, Philippines. The inoculum size of each tested organism was standardized. The standardized inocula were then plated on Mueller-Hinton agar through cotton swabbing and the plates were incubated for 12 h. Six sterilized Whatman No.1 filter paper discs, 6 mm diameter, were immersed in the ethanolic leaf extracts and the discs were then placed on inoculated Mueller-Hinton agar plates with the bacterial strains. The sample plates containing six paper discs were replicated thrice. The sample plates were then incubated at 37°C for 24 h. Zones of inhibition were measured and their corresponding interferences were described following the rating scale of Guevarra (2005).



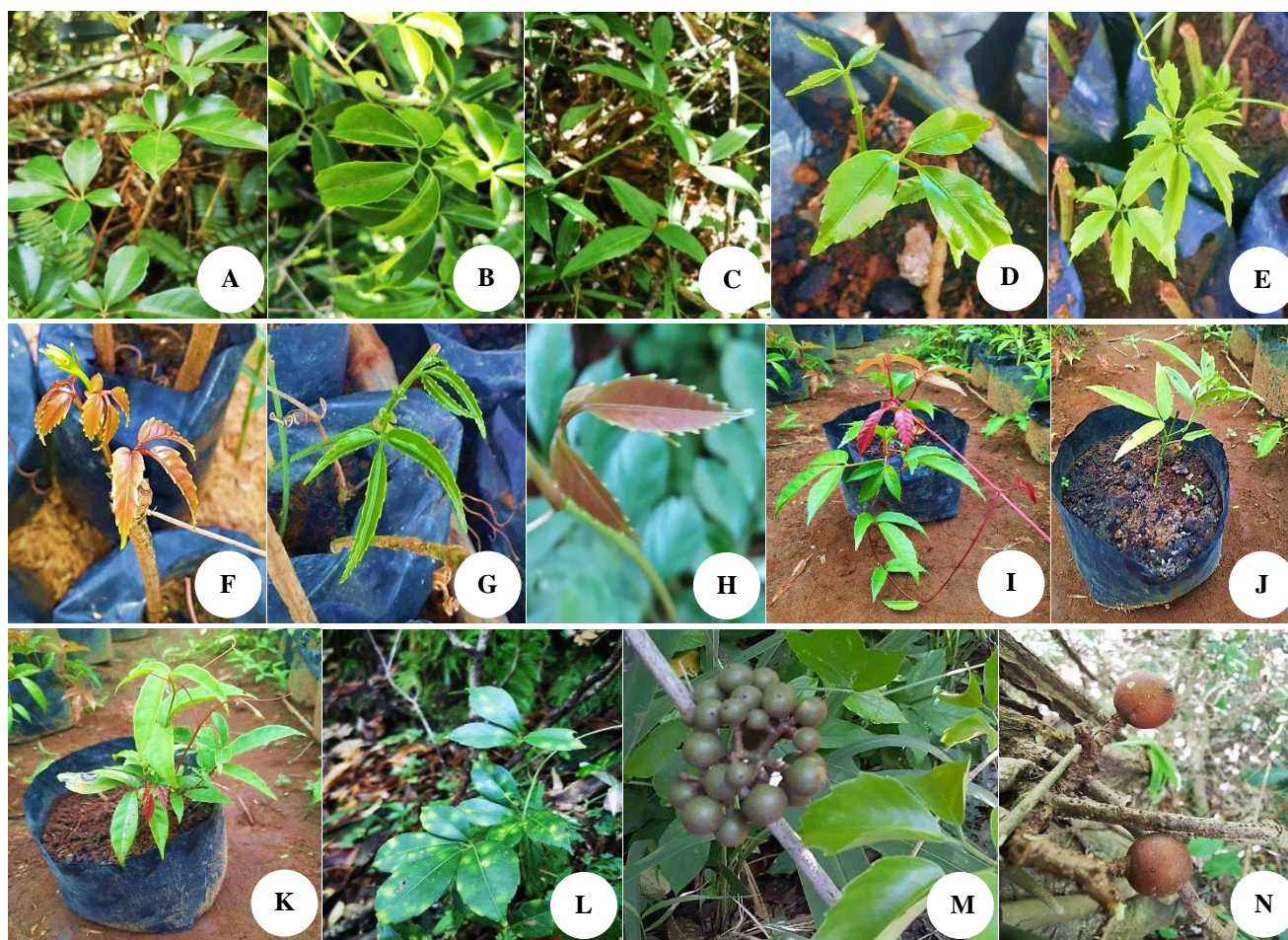
### Nutritional and mineral analysis

Young and mature leaves of the known edible *Tetrastigma* species (*T. harmandii* and *T. loheri*) were analyzed following the procedures of Motsara and Roy (2008). The total nitrogen of the *Tetrastigma* species was determined following the Kjeldahl Jaudber-Gunning method, while the total phosphorus, total potassium, and trace elements (copper, zinc, iron, and manganese) were analyzed using the Vanadomolybdate, Flame Atomic Emission, and Microwave Plasma Atomic Emission Spectroscopy tests, respectively. Moreover, 100 grams of dried leaf tissues were subjected to proximate analysis wherein crude protein content was determined through the Kjeldahl method, while crude fat and crude fiber content were measured through Filter Bag Technique (ANKOM), and calcium content by Titrimetry test. Interpretation of plant tissue analysis results follows the rating scale of Jones (1967).

## RESULTS AND DISCUSSION

### Morphological characteristics of the three *Tetrastigma* species

This study supplements the information provided by Pelser et al. (2016) on the key morphological features of the three Philippine *Tetrastigma* species identified in this study. The old stem shape and appearance, leaf appearance and leaflet shapes, leaf margin, type of venation on the adaxial leaf surface, petiole color, and presence of indumentum in the leaf and petiole were reported in this study. On the other hand, characteristics on tendril structure, number of leaflets, and leaf morphology were the same as those observed by Pelser et al. (2016). Key morphological features are presented in Figure 1 and Table 3.



**Figure 1.** *Tetrastigma* species growing in the northeastern part of Cagayan, Luzon, Philippines, namely *Tetrastigma ellipticum* Merr. (A), *Tetrastigma harmandii* Planch. (B), and *Tetrastigma loheri* Gagnep (C) which were found in various habitats in Cagayan. Newly emerged leaves of *Tetrastigma ellipticum* Merr. (D), and *Tetrastigma harmandii* Planch. (E) were observed during asexual propagation. Newly emerged and young fully opened leaves of *Tetrastigma loheri* Gagnep found in Palaui Island, Santa Ana (F, I) and Mt. Cagua, Sierra Madre Mountain range in Gonzaga (G, H, J, K) Cagayan have different colors as observed during asexual propagation. A unique accession of *Tetrastigma ellipticum* Merr. found in Mt. Cagua forest has dark green mature leaves with visible yellow spots (L). *Tetrastigma harmandii* Planch species in northeastern Cagayan bear young, green, globose (M), and mature, brown, globose fruits (N) during the month of June to October.

**Table 3.** Morphological characteristics of three *Tetrastigma* species growing in various habitats of the northeastern part of Cagayan, Philippines

Scientific name	Key morphological features							
	Old stem shape and characteristics	Leaf morphology and characteristics	Number of leaflets	Leaf margin	Type of venation on the adaxial leaf surface	Petiole color	Leaf and petiole indumentum	Tendrils
<i>Tetrastigma ellipticum</i> Merr.	Rounded, semi-woody to woody stem	Palmately compound leaves with glossy. light to dark green leaflets Light green young leaves Other plants found have yellow spots in the leaves Elliptical leaflets	3,4,5	Serrate	Pinnate	Young and mature leaves have both green petioles	Absent	Forked/ branched tendrils
<i>Tetrastigma harmandii</i> Planch.	Flat, woody stem (others have rounded stem)	Pedately compound leaves with glossy dark green leaflets Light green young leaves Elliptic-oblong leaflets	(3) 5 (7)	Serrate	Cross-venulate	Young and mature leaves have both green petioles	Absent	Unforked/ unbranched/ simple tendrils
<i>Tetrastigma loheri</i> Gagnep.	Rounded, semi-woody to woody stem	Pinnately trifoliate leaves with dark green leaflets Light green to reddish young leaves Lanceolate leaflets	3	Serrate	Pinnate	Young leaves have green to reddish petioles while mature leaves have green petioles	Absent	Unforked/ unbranched / simple tendrils

*Tetrastigma ellipticum* Merr.

The plants were encountered in the forests and near the volcano crater of Mt. Cagua, Sierra Madre Mountain range in Gonzaga, Cagayan. Species found in the other areas of Mt. Cagua with an altitude of 861.97 m asl. had yellow spots in the leaflets. The newly emerged leaves were light green and trifoliate which could be confused with those of *T. loheri* considering that both species were found in the forests of Mt. Cagua. The only difference in the newly emerged young leaves of *T. ellipticum* from *T. loheri* was the broader lamina of its trifoliate leaves unlike the elongated lamina of the trifoliate leaves of *T. loheri* (Figure 1, Table 3). The older stem of the *T. ellipticum* species varied from semi-woody to woody. The species encountered were in their vegetative stage and were not yet flowering or fruiting.

*Tetrastigma harmandii* Planch.

Most of the species found in the municipalities of northeastern Cagayan, except in the municipalities of Buguey, Gonzaga, and Lal-lo, had no flowers. In the three municipalities, some of the plants bore fruits (Figure 1) in June to October. The color of the leaves depended on the location. The plants found in the shaded area had darker green leaves compared to those in exposed areas. Some accessions found in Cabiraon, Gonzaga have light green mature leaves with conspicuous yellow midribs. Also, an accession found in Santa Maria, Lal-lo had unique morphological characters in that the lenticels of the old stem as well as the leaf venation were prominent. It also had undulate, thick, and large leaflets resembling the leaves

of Robusta coffee. *Tetrastigma harmandii* was observed to be the most common *Tetrastigma* species in Cagayan. The leaves were used as a souring agent in cooking and were known to remove the fishy smell of fish-based dishes.

*Tetrastigma loheri* Gagnep.

*Tetrastigma loheri* species in Mt. Cagua (Sierra Madre Mountain range) in Gonzaga, Palau Island (Protected Landscape and Seascape), and Buwacag mountains in Santa Ana were morphologically different in terms of young leaf characteristics. *Tetrastigma loheri* species encountered in the forests and near the volcano crater of Mt. Cagua Sierra Madre Mountain range in Gonzaga had red to light green newly emerged leaves, while species encountered in the mountain forest of Buwacag in Santa Clara, Santa Ana had light green newly emerged leaves (Figure 1, Table 3). However, *T. loheri* growing in mountain forests of Palau Island in Santa Ana had red to reddish-yellow newly emerged leaves. The differences in the leaf color of newly emerged leaves were observed in the nursery when plants were propagated through stem cuttings (Figure 1). Plants found in Mt. Cagua, Buwacag mountains, and Palau Island forests were creeping on the soil and climbing on other plant species. These plants were at the vegetative stage.

**Multivariate analysis of the *Tetrastigma* species growing in northeastern Cagayan**

A Principal Coordinate Analysis (PCoA) was performed to determine the dis(similarity) among the different accessions of *Tetrastigma* species found in

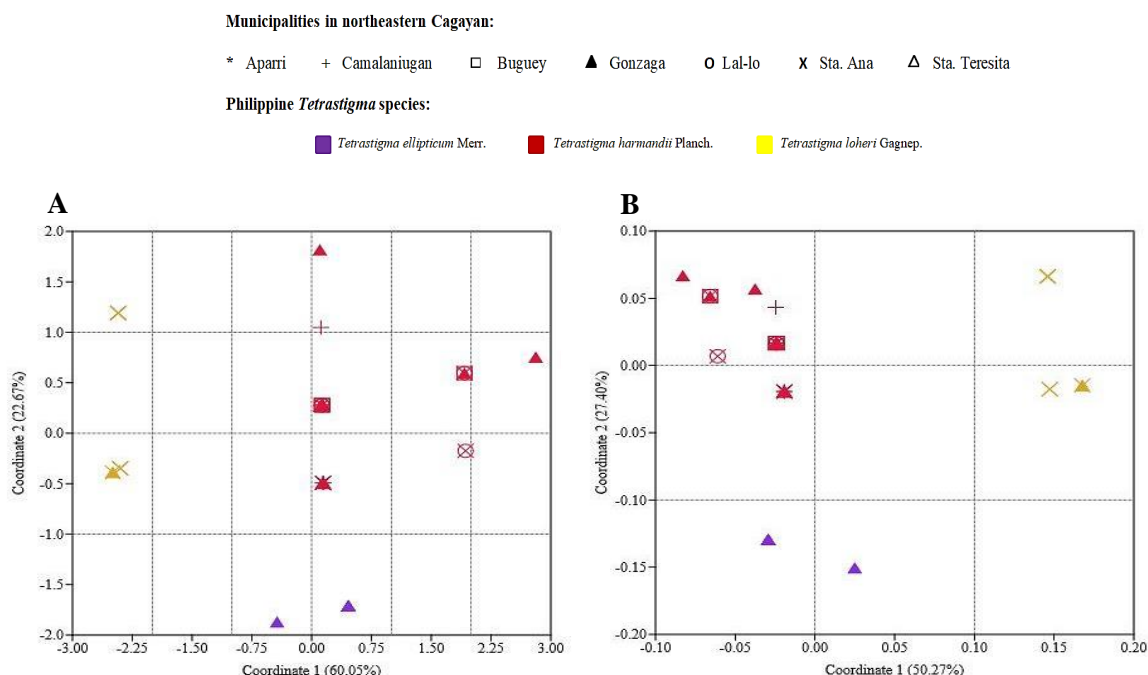
various municipalities of northeastern Cagayan (Figure 2). This was based on the morphological characters, namely old stem shape and characteristics, leaf organization, leaflet number of matured leaves, young leaf color, leaf shape and margin, type of venation, leaf and petiole indumentum, and tendril morphology. Using Euclidean similarity index, coordinates 1 (79.507 eigenvalue) and 2 (30.015 eigenvalue) accounted for 60.05% and 22.67% variations, respectively. Meanwhile, Bray-Curtis similarity index accounted for 50.27% and 27.40% variations for coordinate 1 (0.243 eigenvalue) and coordinate 2 (0.133 eigenvalue), respectively. Scatter plots showed that *T. harmandii* species found in various municipalities of northeastern Cagayan showed more similarity with *T. ellipticum* accessions found in Mt. Cagua, Gonzaga than the *T. loheri* accessions found in Gonzaga (Mt. Cagua) and Sta. Ana (Buwacag mountains and Palaui Island), Cagayan. The similarity between *T. harmandii* and *T. ellipticum* was evident due to some morphological characteristics where both species have compound leaves with green petioles, and more than three, light green, elliptical leaflets as opposed to the trifoliolate leaves with light green to reddish, lanceolate leaflets, and green to reddish petioles of the young leaves of *T. loheri*.

PCoA scatter plots also showed differences among *T. harmandii* and *T. loheri* accessions in different localities. Less similarity was shown in some *T. harmandii* accessions found in Gonzaga due to differences in the old stem shape and characteristics, and leaflet number of matured leaves. On the other hand, *T. loheri* accessions found in Palaui Island forest, Sta Ana were less similar than those

accessions found in Buwacag mountains, Sta. Ana and Mt. Cagua forest, Gonzaga. Less similarity was due to differences in the color of the newly emerged leaves where *T. loheri* accessions found in Palaui Island have reddish-yellow newly emerged leaves as opposed to the light green newly emerged leaves of *T. loheri* accessions found in Buwacag mountains and Mt. Cagua forest. Morphological differences among the accessions within a species may be accounted to the type of habitat or locality where these accessions are growing. These data are important in selecting representative accessions to be included in further *Tetrastigma* investigations or studies. Further studies showing the similarity of the species based on molecular characteristics are suggested.

### Geographical distribution of *Tetrastigma* species and their environments

Three species of Philippine *Tetrastigma* were found in various habitats of northeastern Cagayan, Philippines. In this study, we report the updated preliminary data on diversity, distribution, and growing environment characteristics of the three *Tetrastigma* species growing in northeastern Cagayan, Philippines. *Tetrastigma* species distribution in the Philippines was reported in several studies where the three species were said to be widely distributed in Luzon, Visayas, and Mindanao. Reports on the specific location of distribution for *T. ellipticum* were in Basilan, Panay, and Dinagat while *T. loheri* was found in Negros, Panay, Samar, Mindoro and Palawan.

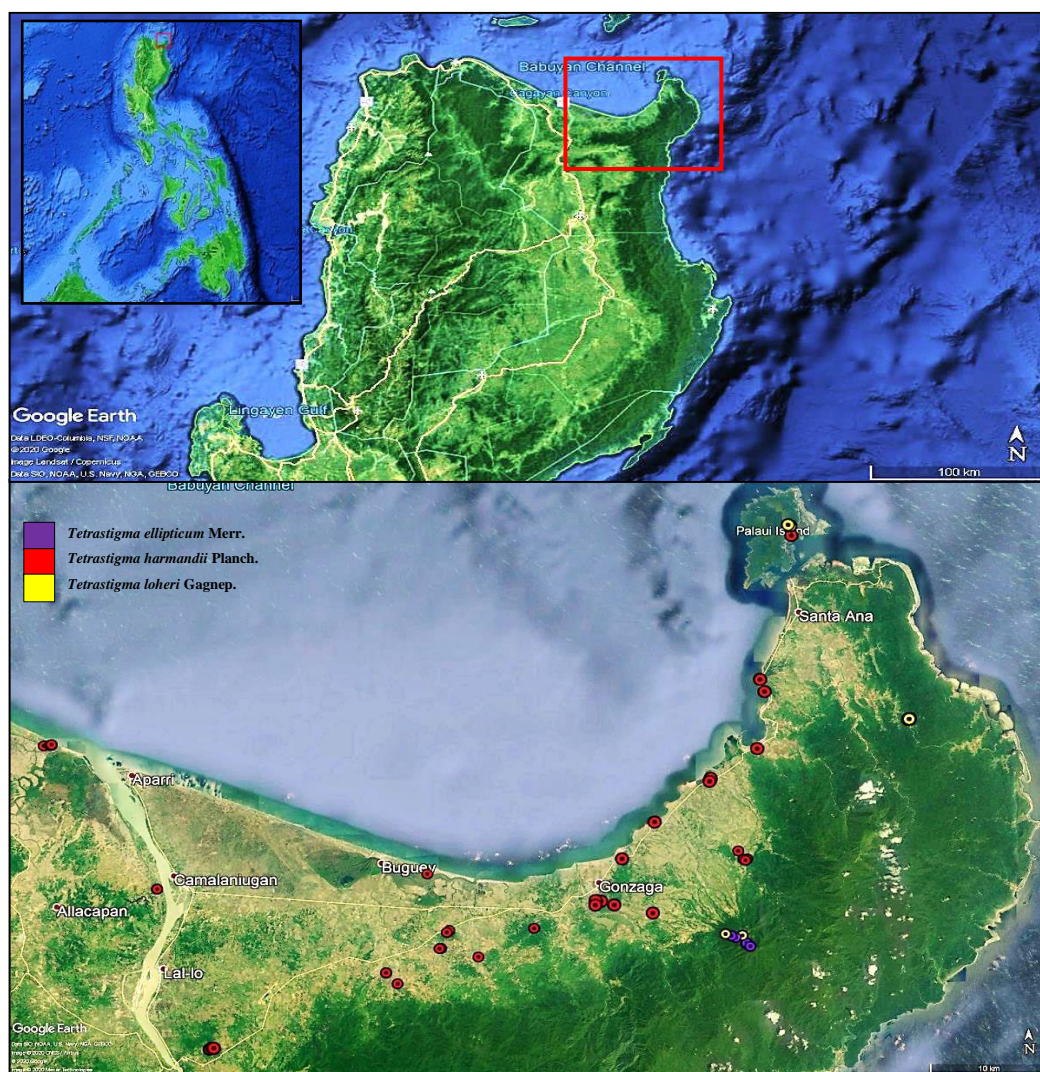


**Figure 2.** Principal Coordinate Analysis (PCoA) scatter plots of three *Tetrastigma* species growing in various municipalities of northeastern Cagayan, Luzon, Philippines, based on morphological traits. PCoA was calculated using PAST (Hammer et al. 2001). PCoA plots were computed based on Euclidean (A) and Bray-Curtis (B) similarity indices, transformation exponent  $c=2$  and eigenvalue scale



Meanwhile, *T. harmandii* was distributed in Mindoro, Panay, and Negros (Wen et al. 2013; Pelser et al. 2016). The only report in Cagayan Province was in Bolos Point where *T. loheri* was found (Pelser et al. 2016). The study has encountered these three species of *Tetrastigma* which were distributed around northeastern Cagayan (Figure 3). This study is the first detailed report on the habitat and growing environment of the three *Tetrastigma* species. Of the 49 *Tetrastigma* species accessions encountered during the study, 36 accessions of *T. harmandii* Planch. species were widespread and found in all municipalities and found growing in various elevations ranging from 18.89 m asl. to 187.15 m asl.. This species was found in various habitats, including residential areas, near coastal areas, nipa plantations, agricultural lands planted with rice, corn, and vegetables, grasslands, near water bodies (rivers, creeks, dam, and freshwater spring), secondary forests, outside the caves, and in the community of Palau Island (Figure 4). However, this species was not found in high elevations

such as in Mt. Cagua forests, summit/grassland, and volcano, Palau Island, and Buwacag mountain forests. Meanwhile, *T. ellipticum* Merr. and *T. loheri* Gagnep. were found in the Mt. Cagua forests with high elevations. *Tetrastigma ellipticum* species were only encountered in Mt. Cagua, Gonzaga, Cagayan (793.08 m asl. to 862.88 m asl.) and were not found in other forests such as in Santa Ana Buwacag mountains and Palau Island mountains wherein *T. loheri* also existed. *Tetrastigma loheri* was found in the forests of Mt. Cagua (698.90 m asl. to 937.86 m asl.), Buwacag mountains, and Palau Island (56.08 m asl. to 156.05 m asl.). *Tetrastigma loheri* and *T. ellipticum* were highland or mountain species. The same observation was reported by Pelser et al. (2016) where these two species grew mostly in various mountains in the Philippines. Moreover, *T. ellipticum* and *T. loheri* were found near the Mt. Cagua volcano crater with high sulfur and high-temperature environment, while *T. harmandii* thrived near coastal areas with saline sandy soil.

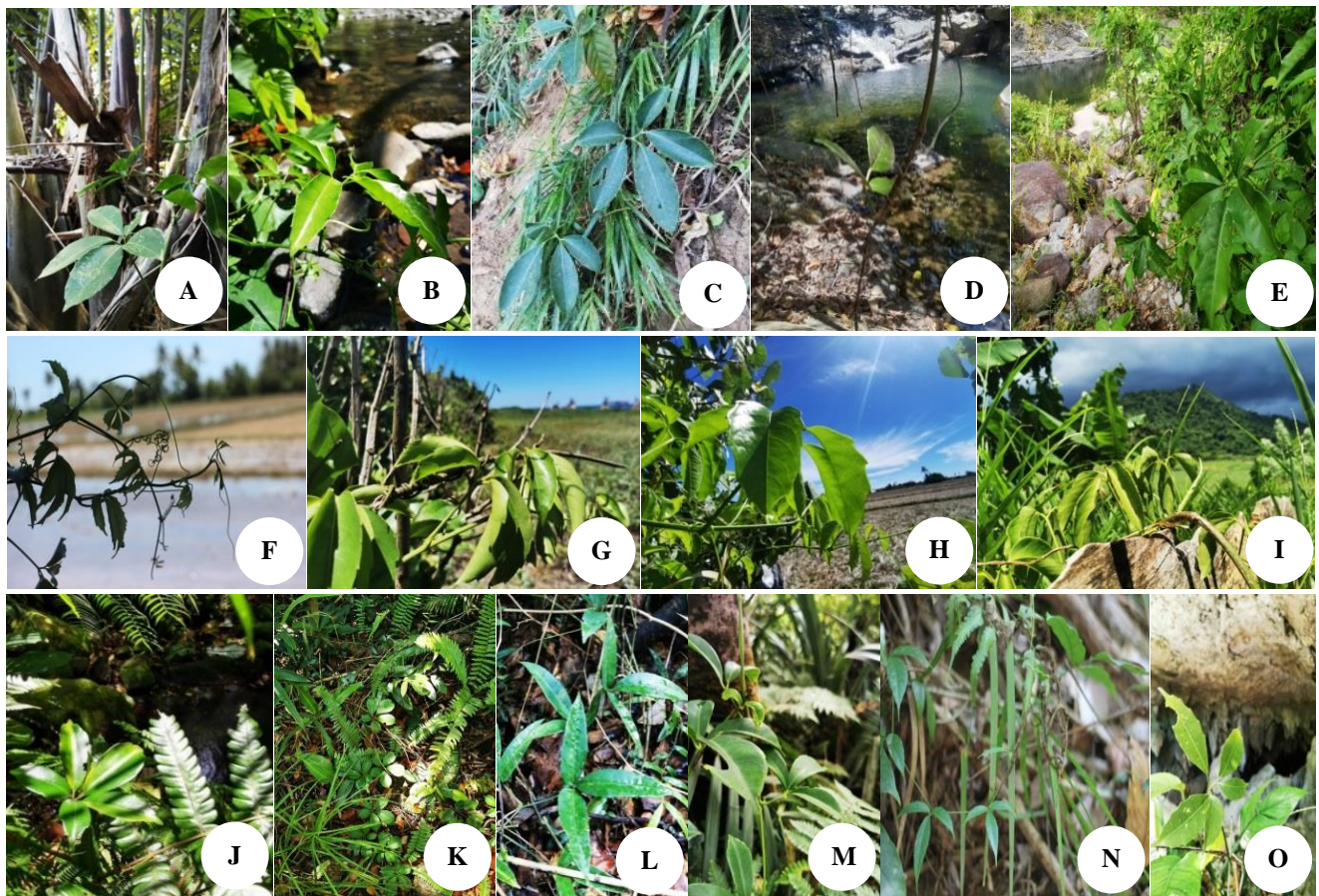


**Figure 3.** Geographical distribution of three *Tetrastigma* species in northeastern part of Cagayan, Luzon, Philippines



The vines of *T. harmandii* were mostly growing on trees such as *Melanolepis multiglandulosa* (Reinw. Ex Blume) Reichb. & Zoll. [Alim], *Macaranga tanarius* (Linn.) Muell.-Arg [Samak], *Gliciridia sepium* (Jacq.) Kunth ex Walp. [Kakawate], *Ficus ulmifolia* Lam. [Oplas], *Ficus nota* (Blanco) Merr. [Tibig], *Antidesma bunius* (L.) Spreng [Bignay], *Hibiscus tiliaceus* Linn. [Malabago], *Kleinhovia hospita* Linn. [Biknong], *Coffea robusta* Linden [Kape], and other trees. Meanwhile, vines of *T. ellipticum* and *T. loheri* were mostly encountered as creeping on the soil with other vegetation and climbing on taller plants such as *Dillenia philippinensis* Rolfe [Katmon], *Shorea contorta* Vidal [Lauan], *Wallaceodendron celebicum* Koord. [Banuyo], *Calamus rotang* Linn. [Rattan], *Diplodiscus paniculatus* Turcz. [Balobo], *Donax*

*canniformis* (G. Forst.) K. Schum. [Darumaka], *Dracontomelon dao* (Blanco) Merr. & Rolfe [Dao], *Ficus nota* (Blanco) Merr. [Tibig], *Hibiscus tiliaceus* Linn. [Malabago], *Diospyros philippinensis* A. DC. [Kamagong], and other plants (Table 4). *T. ellipticum*, *T. harmandii*, and *T. loheri* were known to be distributed in the regions of Luzon, Philippines (Pelster et al. 2016). In this study, we observed that most of the plant species growing within the *Tetrastigma* environments were wild bamboo and wild ferns. Other plant species such as agricultural crops, native and endemic species were also found growing nearby and these were believed to provide a favorable growing environment by providing partial shade to the *Tetrastigma* plants (Table 3).



**Figure 4.** *Tetrastigma* species growing in various habitats in northeastern Cagayan, Luzon, Philippines. *Tetrastigma* species were encountered in various habitats such as nipa plantations (A), creeks (B), spring (C), falls (D), dam (E), Cagayan river (F), near coastal areas (G), agricultural lands (H), grasslands (I), Mt. Cagua river (J), Mt. Cagua forests (K, L), near Mt. Cagua volcano crater (M), Palau Island forests (N), and outside the cave (O). *Tetrastigma harmandii* Planch. (A-C, E-I, O), *Tetrastigma ellipticum* Merr. (J, K), and *Tetrastigma loheri* Gagnep. (D, L, M, N) were found growing in different municipalities of northeastern Cagayan

**Table 4.** Geographical distribution and environment of the three *Tetrastigma* species in the northeastern part of Cagayan, Philippines

Species	Location	Habitats and ecology	Latitude-longitude and elevations (m asl.)	Other plants growing within the <i>Tetrastigma</i> environments
<i>Tetrastigma ellipticum</i> Merr.	Mt. Cagua, Santa Clara, Gonzaga	High elevation mountain forests (793.09-862.89 m asl.), Primary growth forests, Near the Mt. Cagua creek, Near the Mt. Cagua volcano (approx.100 meters below the volcano crater), Above the Mt. Cagua falls	18.21°N, 122.11°E to 18.22°N, 122.10°E 793.08 to 862.88 m asl.	<i>Dillenia philippinensis</i> Rolfe [Catmon/Katmon] <i>Shorea contorta</i> Vidal [Lauan] <i>Wallaceodendron celebicum</i> Koord. [Narang-dawer, Derahm mahogany/Banuyo] <i>Pandanus tectorius</i> Parkinson ex Du Roi [Screw pine/Pandan] <i>Calamus rotang</i> Linn. [Rattan] <i>Dinochloa dielsiana</i> Pilger [Bikal-boboi] <i>Bambusa spp.</i> [Wild bamboo] <i>Asplenium nidus</i> Linn. [Bird's nest fern/pakpak-lauin] [Wild ferns]
<i>Tetrastigma harmandii</i> Planch.	Santa Maria, Lal-lo, Bulala Norte, Aparri, Babaywan Creek, Juaquin Dela Cruz, Camalaniugan, Cagayan river, Camalaniugan, Nangatulan Creek, Villa Cielo, Buguey, Villa Leonora, Buguey, Luga, Santa Teresita, Tabaco Cave, Luga, Santa Teresita, Aridowen, Santa Teresita, Mission, Santa Teresita, Flourishing, Gonzaga, Santa Clara, Gonzaga,	Lowland (18.90-74.07 m asl.), Hills (85.95-181.97 m asl.), Residential area, Near from coastal areas (approx. 5-200 meters away from the sea), Nipa plantations, Agricultural lands/Agroecosystem, Grasslands, Near from rivers, creeks, dam, swamp, spring, and irrigation canals, Outside the cave, Secondary growth forest Island shorelines,	18.13°N, 121.69°E to 18.13°N, 121.70°E 85.95 to 107.59 m asl. 18.37°N, 121.57°E to 18.38°N, 121.58°E 18.89 to 45.11 m asl. 18.26°N, 121.66°E to 35.96 to 41.14 m asl. 18.18°N, 121.84°E to 18.27°N, 121.86°E 46.93 to 53.94 m asl. 18.20°N, 121.90°E to 18.23°N, 121.95°E 43.89 to 74.06 m asl. 18.24°N, 121.99°E to 18.35°N, 122.08°E 18.29°N, 122.10°E 39.93 to 187.15 m asl. 18.37°N, 122.12°E to 18.54°N, 122.15°E 32.92 to 52.12 m asl.	[Dirig] <i>Melanolepis multiglandulosa</i> (Reinw. Ex Blume) Reichb. & Zoll. [Alim/Alem] <i>Gnetum gnemon</i> Linn. [Bago] <i>Macaranga tanarius</i> (Linn.) Muell.-Arg [Elephant's ear/Binunga,Samak] <i>Antidesma bunius</i> (L.) Spreng [Currant tree/Bignay] <i>Antidesma ghaesembilla</i> Gaertn [Black Currant tree/Arosep,Binayuyo] <i>Phragmites vulgaris</i> (Lam.) Trin. [Common Reed/Tambo,Tanobong] <i>Pterocarpus indicus</i> Willd. [Rosewood/Narra] <i>Kleinhovia hospita</i> Linn. [Timanga tree/Biknong] <i>Hibiscus tiliaceus</i> Linn. [Sea Rosemallow/Malabago] <i>Donax canniformis</i> (G. Forst.) K. Schum. [Common donax/Darumaka] <i>Harpullia arborea</i> (Blanco) Radik. [Tulip wood tree/Uas] <i>Spathodea campanulata</i> P.Beauv. [African tulip tree/Sirit-sirit] <i>Ficus nota</i> (Blanco) Merr. [Sacking tree/Tibig] <i>Ficus septica</i> Blanco [Hauili fig tree/Hauili, Ria-ria] <i>Ficus ulmifolia</i> Lam. [Isis, Oplas] <i>Ficus benjamina</i> Linn. [Weeping fig/Balete] <i>Gmelina arborea</i> Robx. [White teak/Gmelina] <i>Swietenia mahogani</i> (L.) Jacq. [Mahogany] <i>Ceiba pentandra</i> (L.) Gaertn [White silk cotton tree/Kapok] <i>Eucalyptus globulus</i> Labill [Blue gum tree/Eucalyptos] <i>Areca catechu</i> L. [Areca nut palm/Bunga, Boa] <i>Syzygium cumini</i> (L.) Skeels [Java plum/Duhat] <i>Saribus rotundifolius</i> (Lam.) Blune [Fan palm/Anahaw] <i>Jatropha curcas</i> Linn. [Physic nut tree/Tubang-bakod] <i>Ehretia microphylla</i> Lam. [Wild tea/Tsaang gubat] <i>Piper sarmentosum</i> Roxb. [Betel leaf/Gawed] <i>Pandanus tectorius</i> Parkinson ex Du Roi [Screw pine/Pandan] <i>Nypa fruticans</i> Wurm. [Sasa/Nipa] <i>Morus alba</i> Linn. [Mulberry/Morera] <i>Bambusa spp.</i> [Bamboo] <i>Coffea robusta</i> Linden.[Robusta Coffee/Kape] <i>Musa sp.</i> [Banana/Saging] <i>Cocos nucifera</i> (L.) [Coconut/Niyog]

<i>Tetrastigma harmandii</i> Planch..	Calayan, Gonzaga,  Ipil, Gonzaga  Baua, Gonzaga,  Cabiraoan, Gonzaga,  Casambalangan, Santa Ana  Racat, Santa Ana  Zinungan, Santa Ana,  Punta Verde, Palaui Island, San Vicente, Santa Ana,				<i>Artocarpus heterophyllus</i> Lam [Jackfruit/Langka] <i>Mangifera indica</i> L. [Mango/Mangga] <i>Carica papaya</i> L. [Pawpaw/Papaya] <i>Sandoricum koetjape</i> (Burm.f.) Merr.[Lolly fruit/Santol] <i>Psidium guajava</i> Linn. [Guava/Bayabas] <i>Tamarindus indica</i> Linn. [Tamarind/Sampalok] <i>Gliciridia sepium</i> (Jacq.) Kunth ex Walp. [St. Vincent Plum/Kakawate] <i>Tabernaemontana pandacahui</i> Poir. [Banana bush/Kampupot] <i>Chromolaena odorata</i> (L.) R.M. King & H. Rob. [Siam weed/Hagonoy] <i>Leucaena leucocephala</i> (Lam.) de Wit [Lead tree/ Ipil-ipil] <i>Imperata cylindrica</i> (L.) Raeusch [Cogon grass, Speargrass/Kogon,Pan-au] <i>Dinochloa dielsiana</i> Pilger [Bikal-boboi] <i>Ipomoea aquatica</i> Forsk. [Water spinach/Kangkong] <i>Panicum maximum</i> Jacq.[Guinea grass] <i>Passiflora foetida</i> Linn. [stinking passionflower/Prutas-Baguio] [Marapapaya] <i>Capsicum</i> sp. [Wild capsicum] <i>Solanum torvum</i> Sw. [Turkey berry, Devil’s Fig/Tandang-aso] <i>Colocasia esculenta</i> Linn. [Taro/Gabi] <i>Tradescantia spathacea</i> Sw. [Moses-in-the-cradle, boat lily/Bangka-bangkaan] <i>Monstera deliciosa</i> Liebm. [Swiss cheese plant, split-leaf philodendron] <i>Mucuna pruriens</i> (L.) DC. [Velvet bean/Sabawil] <i>Centrosema pubescens</i> Benth. [Butterfly pea, Centro/Dilang-butiki] <i>Mimosa pudica</i> L. (Laajvanti) [Sensitive plant/Makahiya] [Wild ferns]
<i>Tetrastigma loheri</i> Gagnep.	Mt. Cagua, Santa Clara, Gonzaga  Buwacag Falls, Santa Clara, Santa Ana  Punta Verde, Palaui Island, San Vicente, Santa Ana	High elevation mountain forest (698.91-937.87 m asl.), Mid elevation mountain forest (123.14-156.06 m asl.), Low elevation mountain forest (56.08-86.87 m asl.), Primary growth forests, Near the Mt. Cagua volcano (approx. 100 meters above the volcano crater), Below the peak of Mt. Cagua, Near bodies of water (river, falls), Island,	18.22°N, 122.09°E to 18.22°N, 122.10°E 698.90 to 937.86 m asl. 18.39°N, 122.23°E to 18.55°N, 122.15°E 56.08 to 156.05 m asl.		[Daer] <i>Diplodiscus paniculatus</i> Turcz.[Bagobo, Balobo] <i>Donax canniformis</i> (G. Forst.) K. Schum. [Common donax/Darumaka] <i>Shorea contorta</i> Vidal [White lauan] <i>Dracontomelon dao</i> (Blanco) Merr. & Rolfe [Pacific Walnut/Dao] <i>Ficus nota</i> (Blanco) Merr. [Sacking tree/Tibig] <i>Hibiscus tiliaceus</i> Linn. [Sea Rosemallow/Malabago] <i>Diospyros philippinensis</i> A. DC. [Kamagong gubat] <i>Pterocarpus indicus</i> Willd. [Rosewood/Narra] <i>Mangifera indica</i> L. [Mango/Mangga] <i>Artocarpus</i> sp. [Artocarpus] <i>Citrus limon</i> (L.) Burm. [Lemon] <i>Calamus rotang</i> Linn. [Rattan] <i>Dinochloa dielsiana</i> Pilger [Bikal-boboi] <i>Bambusa</i> spp. [Wild bamboo] <i>Thysanolaena maxima</i> (Roxb.) [Tiger grass] <i>Caladium</i> sp. [Wild caladium] <i>Begonia</i> sp. [Wild begonia] <i>Spathoglottis plicata</i> Blume [Wild ground orchids] [Wild ferns]

Note: Observations were done on June-October 2020. Names inside brackets [ ] are common names of plant species.

### Soil characteristics of the *Tetrastigma* species habitats

The chemical composition of soils where the *Tetrastigma* species grew is varied (Table 5). *Tetrastigma ellipticum* species grew in soils with extremely acidic pH with medium to high organic matter content, low to medium phosphorus content, medium to high potassium content, medium to high copper content, very low to low zinc content, very high iron content, and medium to very high manganese content. On the other hand, *T. harmandii* species grew in soils with strongly acidic to slightly acidic pH with low to medium organic matter content, high to excessive phosphorus content, high potassium content, low to high copper content, very low to medium zinc content, high to very high iron and manganese contents. *Tetrastigma loheri* species grew in soils with extremely acidic to moderately acidic pH with low to high organic matter content, low to medium phosphorus content, high to excessive potassium content, medium to very high copper content, very low to high zinc content, very high iron and manganese contents.

To compare the species, *T. ellipticum* and *T. loheri* grew in soils with very low pH, while *T. harmandii* grew in soils with higher pH. The soils where *T. ellipticum* and *T. loheri* were found also had higher organic matter content than those of *T. harmandii*. However, the soils where *T. harmandii* grew contained the highest phosphorus while those of *T. loheri* contained the highest potassium. In terms of the micronutrient content, the soils where *T. loheri* was found had the highest copper, zinc, and moderately high manganese content while *T. ellipticum* grew in soils with the highest iron content but lowest copper, zinc, and manganese contents.

### Phytochemical screening of the *Tetrastigma* species

Phenols, tryptophan, and tannins were commonly present while steroids were commonly absent in the three *Tetrastigma* species (Table 6). Secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, anthocyanin, and tannins were detected in the leaves of *T. ellipticum*. Leaves of *T. harmandii* and *T. loheri* contain phenols, terpenoids, and tannins. However, saponins were not detected in *T. loheri* species. *Tetrastigma ellipticum* differed from other species due to the presence of alkaloids, flavonoids, and anthocyanins, while *T. loheri* and *T. harmandii* lack the aforementioned secondary metabolites. Also, *T. ellipticum* and *T. loheri* lack saponins. Among the three *Tetrastigma* species, only the phytochemical property of *T. harmandii* was investigated (Agngaryngay et al. 2015, unpublished data). With the above-mentioned findings, the extraction and utilization of secondary metabolites in the *Tetrastigma* species for pharmaceutical and industrial

purposes would be easier since their potential sources were already identified.

### Antibacterial properties of the *Tetrastigma* species

The antibacterial properties of the three *Tetrastigma* species in northeastern Cagayan against various gram-negative and gram-positive pathogenic bacteria are presented in Table 7. This is the first study on the antibacterial profiles of the three Philippine *Tetrastigma* species. Among the three species, only the *T. harmandii* demonstrated an inhibitory effect on the growth of some bacterial strains such as *Staphylococcus aureus* and *Klebsiella aerogenes* with 10 mm and 9 mm mean zones of inhibition, respectively. Antibacterial activity of the leaf ethanolic extract of *T. harmandii* demonstrated a partially active reaction against *Staphylococcus aureus* and a least active reaction in *Klebsiella aerogenes*. Mountain forest species of *Tetrastigma* did not show any antibacterial activity against the tested microorganisms. Among the three *Tetrastigma* species, only the *T. harmandii* contained saponins which were reported to have antibacterial mechanism against *Staphylococcus aureus* (Avato et al. 2006; Khan et al. 2018).

### Nutritional and mineral profiles of the edible *Tetrastigma* species

*Tetrastigma harmandii* and *T. loheri* species are utilized for food because of their edible sour leaves (Morton and Collectanea, 1968). This study is the first report on the nutritional profiles of these two edible species (Table 8). *Tetrastigma harmandii* was found to be deficient in nitrogen, low phosphorus, high potassium, and sufficient calcium, manganese, iron, zinc, and copper contents. On the other hand, *T. loheri* was found to be deficient in nitrogen and phosphorus, high potassium, low calcium, sufficient manganese, iron, and zinc, and high copper content.

Per 100 g dry weight of *Tetrastigma* leaves, *T. loheri* contained higher crude protein, crude fat, and crude fiber than *T. harmandii* with 6.91%, 0.94%, and 22.2% more crude protein, crude fat, and crude fiber, respectively. In contrast, *T. harmandii* contained more macronutrients and micronutrients compared to *T. loheri*. *Tetrastigma harmandii* contained 0.72% more nitrogen, 0.04% more phosphorus, 0.10% more calcium, 3.0 ppm higher manganese, 84.0 ppm more iron, and 19.0 ppm more zinc content than *T. loheri*. However, *T. loheri* contained 0.26% more potassium and 37.0 ppm more copper than *T. harmandii*.



**Table 5.** Soil chemical characteristics of the *Tetrastigma* species growing environment.

Species	Soil chemical characteristics*							
	pH	OM (%)	Available phosphorus (ppm)	Available potassium (ppm)	Trace elements (ppm)			
					Cu	Zn	Fe	Mn
<i>T. ellipticum</i> Merr.	3.17-4.44 ± 0.401	3.85-4.0 ± 0.050	7.90-14.0 ± 1.845	190.0-288.0 ± 28.449	0.44-1.76 ± 0.070	0.38-0.60 ± 0.416	19.20-126.40 ± 1.097	2.40-6.20 ± 31.085
<i>T. harmandii</i> Planch.	5.25-6.71 ± 0.428	0.41-3.99 ± 1.059	20.80-100.0 ± 25.668	296.0-707.0 ± 128.686	0.28-2.28 ± 0.282	0.48-1.38 ± 0.616	7.60-26.20 ± 9.565	5.20-37.60 ± 5.370
<i>T. loheri</i> Gagnep.	4.34-6.46 ± 0.676	1.79-4.02 ± 0.712	2.90-16.40 ± 3.962	311.0-815.0 ± 152.998	0.72-3.90 ± 0.973	0.42-3.46 ± 0.940	10.80-47.80 ± 7.545	6.20-31.40 ± 12.170

Note: n: 5, mean ± Std. Error

**Table 6.** Phytochemical screening of three *Tetrastigma* species leaf crude extracts

Species	Plant Secondary Products*							
	ALK	FLV	PHE	TRP	ANT	TNS	STD	SPN
<i>Tetrastigma ellipticum</i> Merr.	+	+	+	+	+	+	-	-
<i>Tetrastigma harmandii</i> Planch.	-	-	+	+	-	+	-	+
<i>Tetrastigma loheri</i> Gagnep.	-	-	+	+	-	+	-	-

Note: \*ALK: alkaloids, FLV: flavonoids, PHE: phenols, TRP: terpenoids, ANT: anthocyanin, TNS: tannins, STD: steroids, SPN: saponins

**Table 7.** Antibacterial activity of leaf ethanolic extracts of three *Tetrastigma* species

Species	Zone of inhibition (mm)*						
	SA	EC	PA	EF	KP	KA	AB
<i>Tetrastigma ellipticum</i> Merr.	6.0	6.0	6.0	6.0	6.0	6.0	6.0
<i>Tetrastigma harmandii</i> Planch.	10.0	6.0	6.0	6.0	6.0	9.0	6.0
<i>Tetrastigma loheri</i> Gagnep.	6.0	6.0	6.0	6.0	6.0	6.0	6.0

Note: Control (distilled water): 6.0 mm, n = 3, \*(< 10 mm) may be expressed as inactive, (10-13 mm) partially active, (14-19 mm) active, (> 19 mm) very active. SA: *Staphylococcus aureus*, EC: *Escherichia coli*, PA: *Pseudomonas aeruginosa*, EF: *Enterococcus faecium*, KP: *Klebsiella pneumoniae*, KA: *Klebsiella aerogenes*, AB: *Acinetobacter baumannii***Table 8.** Nutritional and mineral composition of *Tetrastigma harmandii* and *T. loheri* growing in northeastern Cagayan, Philippines

Species	Nutritional and mineral content**										
	CP (%)	CF (%)	CFb (%)	N (%)	P (%)	K (%)	Ca (%)	Mn (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)
<i>Tetrastigma harmandii</i> Planch.	3.80	1.37	5.20	1.99	0.18	3.28	0.30	28.0	207.0	41.0	9.0
<i>Tetrastigma loheri</i> Gagnep.	10.71	2.31	27.40	1.27	0.14	3.54	0.20	25.0	123.0	22.0	46.0

Note: \*\*CP: crude protein, CF: crude fat, CFb: crude fiber

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