

***Tetrastigma fruticosum*, a new species of Vitaceae from limestone karst in central Laos**

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Abstract

A new species of *Tetrastigma*, *T. fruticosum* (Vitaceae), from the limestone karst in Khammouane Province, central Laos, is described. The new species can be distinguished from the previously known species in the genus by a combination of its shrubby habit, absence of tendrils, pedately 5-foliate leaves (rarely 4, 6, or 7-foliate) with shallowly emarginated leaflet, and 1-seeded oblong-ellipsoid fruits. A description, photographs, vernacular name and preliminary conservation status are provided for it.

Key words: Cayratieae, endemic, Indochina, phylogeny, Southeast Asian flora, Vitales

Introduction

Tetrastigma (Miquel 1863: 72) Planchon (1887: 423) is a genus comprising over 130 species, distributed in tropical and subtropical Asia with a few extending to Australia (Latiff 1983, Ren & Wen 2007, Peng *et al.* 2021, POWO 2024). The genus is characterized by its dioecious tetramerous flowers with 4-lobed stigmas (Jackes 1989, Trias-Blasi *et al.* 2020, Peng *et al.* 2021). In Laos, 23 species of the genus *Tetrastigma* have been enumerated so far (Gagnepain 1944, Newman *et al.* 2017 onwards, Peng *et al.* 2021).

During our field surveys in limestone karst of the Khammouane province, central Laos in 2022–2024, an interesting species of *Tetrastigma* exhibiting shrubby habit without tendrils was collected. In this study, we examined the details of its morphology and phylogenetic relationship using the noncoding internal transcribed spacer regions (ITS) to compare with known taxa (Fu *et al.* 2011). Based on the results, it is confirmed to be a hitherto-undescribed taxon and we here describe this plant as a new species, *Tetrastigma fruticosum* Soulad., Phonep. & Tagane, as in the taxonomic treatment below.

Materials and methods

Morphological observation

The validity of the new species was verified by detailed comparisons with morphologically similar species through literature review (Gagnepain 1912, 1944, 1950, Hô 2003, Ren & Wen 2007, Kochariphat *et al.* 2014, 2016, Balachandran *et al.* 2017, Trias-Blasi *et al.* 2020), dry specimens from the herbaria BKF, FOF, KAG, TNS, and VNM (Thiers continuously updated), as well as digitized plant specimens available on the web (e.g. JSTOR Global Plants <https://plants.jstor.org/>).

DNA extraction

Total DNA was extracted from silica-gel dried leaves collected in the field. DNA extraction was performed by a 2× CTAB protocol modified from Doyle & Doyle (1987). Amplification and sequencing of the Internal Transcribed Spacer (ITS) were performed with the primer pairs 51NT and 26S1, according to published protocols (Kozoff *et al.* 1998, Clement *et al.* 2004).

Phylogenetic analysis

In total 13 accessions of *Tetrastigma* were included in the phylogenetic analysis using DNA barcoding regions of ITS (754 bp) (Appendix Table 1). *Ampelocissus indica* (Linnaeus 1753: 202) Planchon (1884: 375), *A. tomentosa* (Roth 1819: 318) Planchon (1884: 375), *Cayratia cardiophylla* Jackes (1987: 371) and *C. pedata* (Lamarck 1783: 31) Jussieu ex Gagnepain (1911: 346) were used as outgroups. Sequences except the new species were obtained from GenBank, and their accession numbers are shown in Appendix Table 1. The sequence alignment was performed by ClustalW with default parameter implemented in MEGA11 (Tamura *et al.* 2021).

The Neighbor-joining methods (Saitou and Nei 1987) with Maximum Composite Likelihood distance matrix (Tamura *et al.* 2004) implemented in MEGA11 was used to construct the phylogenetic tree. Confidence values for individual branches were determined by bootstrap analysis with 10,000 repeated samplings of the data.

Results and discussion

The new species is distinguished from previously known species of *Tetrastigma* and its allied genus by a combination of shrubby habit, absence of tendril, pedately 5-foliolate leaves, shallowly emarginate leaflet apex, and 1-seeded oblong-ellipsoid fruits (Figs. 1, 2).

The Neighbor-joining tree based on ITS showed that the new species is located within the clade of *Tetrastigma* species and separated from each species used in this study, with a sister relationship to the clade including *T. campylocarpum*, *T. sulcatum*, *T. lanyuense*, and *T. harmandii* (Fig. 3).

Based on these results, it was clear this is an undescribed species that belongs to *Tetrastigma*, which we formally described below.

Taxonomy

Tetrastigma fruticosum Soulad., Phonap. & Tagane, *sp. nov.* (Figs 1, 2)

TYPE:—LAOS. Khammouane Province, Khounkham District, The Rock Viewpoint, Phou Pha Marn, in semi-shaded limestone karst, 18.177560°N, 104.48400°E, 455 m elev., 7 Sep. 2023, young fr., Tagane *et al.* Z534 (holotype: FOF [FOF0005255!]; isotypes KAG [KAG186966!, KAG187205!], VNM!).

Tetrastigma fruticosum is distinct from all other species in the genus by its shrubby habit to 6 m tall, absence of tendril, pedately 5-foliolate leaves with leaflets shallowly emarginate at apex, and 1-seeded oblong-ellipsoid fruits. Among the species of *Tetrastigma* in the Indochina Peninsula, *T. fruticosum* is most similar to *T. calcicola* Kochariphat & Trias-Blasi in Kochariphat *et al.* (2016: 4) known

from an open limestone mountain in northern Thailand, in leaf shape and size, but clearly distinguished by the above diagnostic characters.



FIGURE 1. Holotype of *Tetrastigma fruticosum* Soulad., Phonep. & Tagane (*Tagane et al.* Z534, FOF [FOF0005255]).

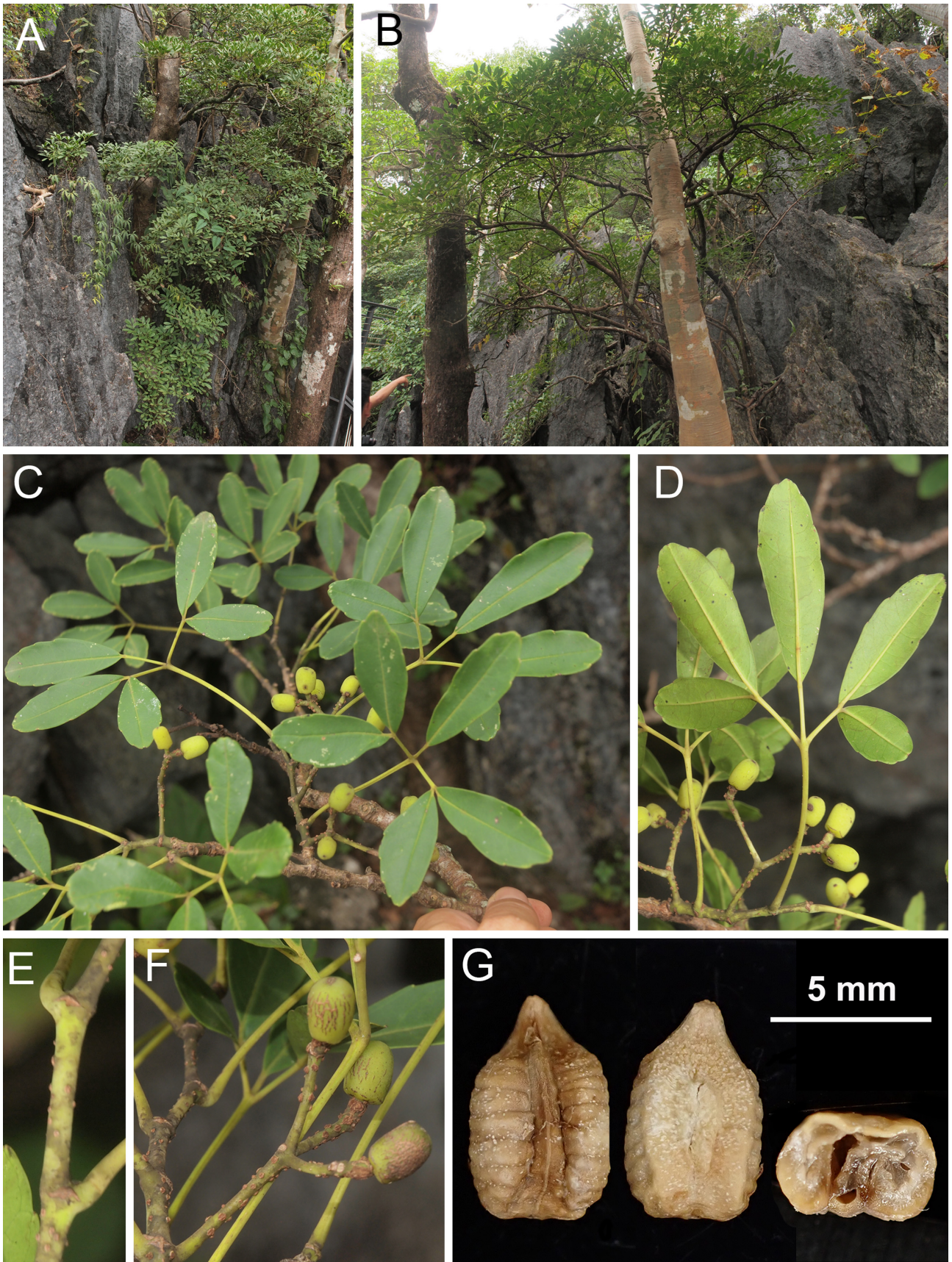


FIGURE 2. *Tetrastigma fruticosum* Soulad., Phonep. & Tagane. A, B. Habit. C. Fruiting branch. D. Abaxial leaf surface. E. Portion of current year branch. F. Infructescence. G. Seeds (from Tagane et al. Z534, KAG). Photographed by S. Tagane.

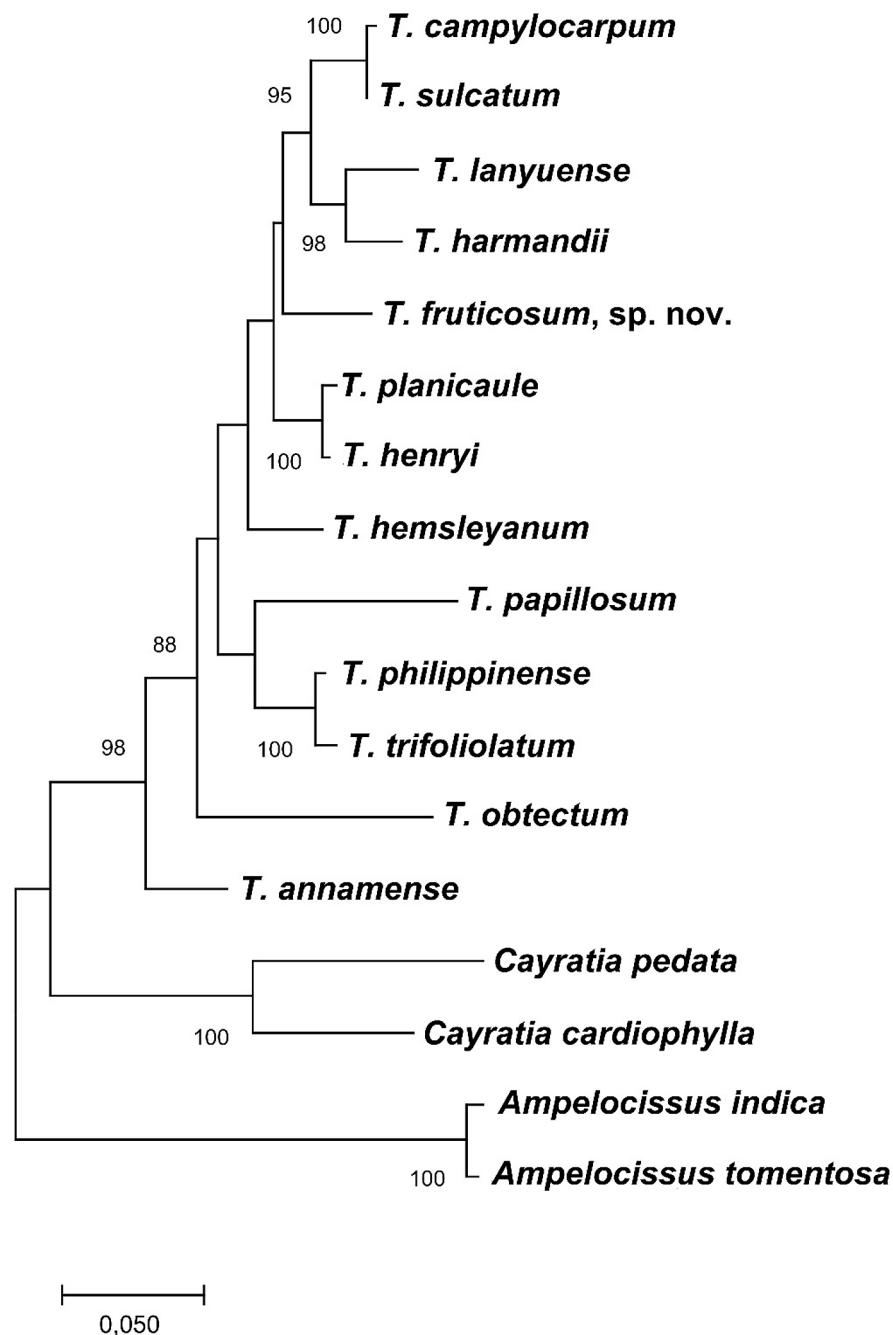


FIGURE 3. Neighbor-Joining tree of *Tetrastigma* species based on internal transcribed spacer regions (ITS). Branches are labelled with bootstrap support (% of 10,000 replicates, shown only higher than 85).

Plants shrub or tree-like shrub, 2.5–6 m tall, up to 10 cm d.b.h. **Stems** terete, bark grayish brown, corky. Young twigs terete, glabrous, lenticellate, yellowish green *in vivo*, blackish brown *in sicco*, old twigs grayish brown *in sicco*, with ridges; tendrils absent. **Stipules** very broadly ovate, 1–1.2 × 2.8–3 mm, glabrous. **Buds** ovate, apex acute. **Leaves** compound, pedately 5-foliolate, rarely 4, 6 or 7-foliolate; petiole 4.1–7.7 cm long, glabrous, base slightly pulvinate; leaflets thinly coriaceous; terminal leaflet petiolule 0.9–1.7 cm long, glabrous, terminal leaflet blade elliptic-obovate, oblong-obovate, 4.2–6.2 × 1.4–2 cm, apex slightly emarginate, margin crenate, with (2–)3–5 teeth on upper 2/3, base acute to cuneate, petiolules of lateral leaflets complex 0.8–2.1 cm long, glabrous, lateral leaflet blades elliptic, elliptic-obovate, obovate, 1.6–5.5 × 0.9–2.1 cm, apex and margin as for terminal leaflet, base cuneate, asymmetrical; midrib sunken adaxially, prominent abaxially, secondary veins 4–6 pairs, prominent abaxially, tertiary veins inconspicuous adaxially, reticulate, slightly prominent abaxially. **Inflorescences and flowers** not seen. **Infructescence** compound cyme, up to 5.2 cm long, 6.5 cm in diam., peduncle 0.9–3.2 cm long, blackish brown, glabrous, lenticellate. **Berries** oblong-ellipsoid, 0.9–1.1 cm long, 0.5–0.7 cm in diam., surface smooth, yellowish green, 1-seeded. **Seeds** oblong-

ellipsoid, 7–8 × 4.5–5 mm, yellowish brown to reddish brown, testa slightly transversely rugulose when young, smooth when mature, adaxial surface with a Y-shaped furrow, abaxial surface with an oblong-elliptic chalaza, endosperm M-shaped in cross-section.

Additional specimen examined:—LAOS. Khammouane Province: Khounkham District, the Rock Viewpoint Phou Pha Marn, 18.177497°N, 104.483794°E, 446 m elev., 2 July 2022, *Souladeth et al. PPM15* (FOF); same locality, 18.17689°N, 104.48409°E, 405 m elev., 19 March 2024, with fruits, *Tagane et al. Z1202* (FOF, KAG [KAG187601, KAG187602], VNM); Gnommalat District, Pha Katai trail, 17.5529°N, 105.1641°E, 173 m elev., 5 September 2024, sterile, *Tagane et al. Z1636* (FOF, KAG [KAG188301], VNM); Hinboun District, walking trail on limestone hills nearby Khoun Kongleng, 17.64508°N, 104.81160°E, 203 m elev., 6 September 2024, sterile, *Tagane et al. Z1703* (FOF, KAG [KAG188366], VNM).

Distribution:—Laos (Khammouane Province).

Habitat:—Open to semi-shaded limestone karst, 170–460 m elev.

Phenology:—Fruiting from July to March.

Etymology:—The specific epithet *fruticosum* refers to the shrubby habit of the new species.

Vernacular name:—Ton Khao Poun (ຕົ້ນໄຂ່ປູນ; Lao), suggested here. Ton in Lao refers to a shrubby habit, Khao Poun refers to common name for *Tetrastigma* in general.

GenBank accession no.: —PP920557 (ITS; sequenced from *Tagane et al. Z534*).

Preliminary conservation assessment:—Endangered (EN). Currently *Tetrastigma fruticosum* is known only from three localities in Khammouane Province, central Laos, where the above specimens collected. We observed about 30 to 50 mature individuals in each location. The Area of Occupancy (AOO) is 12 km² and the Extent of Occupancy (EOO) is calculated as 958.1 km² using GeoCAT (Bachman *et al.* 2011). The species is considered to be endemic to the limestone karst in this area, which is widely seen and mostly located within Phou Hin Phoun National Protected Area. As the topography is very steep and sharp (Fig. 2A), the habitat of *T. fruticosum* is likely stable, but more or less negative impact may be suffered, such as limestone mining, dam construction and construction for tourist attraction. Given this situation, it is assessed as Endangered (EN) according to IUCN criteria B1a, b(iii), 2a, b(iii) (IUCN 2024).

Note:—Among the *Tetrastigma* species in Laos, *T. fruticosum* may be similar to *T. pachyphyllum* (Hemsley 1886: 135) Chun (1940: 235) in its leaf shape and size, but differs in its shrubby habit (vs. woody climber in *T. pachyphyllum*) and smaller and oblong-ellipsoid berries 0.9–1.1 × 0.5–0.7 cm (vs. globose, (1–)1.4–1.8 cm in diam.).

Limestone areas are known to have unique ecosystems and plant diversity due to their alkaline soils and complex geographic features (Clements *et al.* 2006, Maneeanakekul & Sookchaloem 2012). Most species of *Tetrastigma* are dominant climbers in rainforests and subtropical forests and only a few species inhabit lowland shrublands or rocky mountain cliffs (Jackes 1989, Ren & Wen 2007). The shrubby habit of *T. fruticosum* (Fig. 2A, B) is considered to be an adaptation of the severe environment of open and rocky limestone karst of the Phou Pha Marn. This might represent a good example characterizing the endemism of this area. Indeed, several endemic plant species were reported from this limestone and its surrounding areas (Newman 2008, Haevermans *et al.* 2020, Phonepaseuth *et al.* 2021, Vongthavone *et al.* 2024). In addition to these plants, we collected several taxa in various groups including Apocynaceae, Araliaceae, Gesneriaceae, Malvaceae, Melastomataceae, and Phyllanthaceae, which did not match any previously known species. Further botanical inventories and subsequent taxonomic studies are needed to elucidate the overall flora of the limestone area in central Laos.

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Appendix Table 1. Taxon sampling with voucher information and GenBank accession numbers.

Taxon	Voucher	Locality	ITS
<i>Ampelocissus indica</i> (L.) Planch.	-	-	KF544857
<i>Ampelocissus tomentosa</i> (Roth) Planch.	-	-	KF544858
<i>Cayratia cardiophylla</i> Jackes	<i>Chen & Lu 160</i> (PE)	Indonesia, cult. in Bogor Botanical Garden	MT995959
<i>Caryatia pedata</i> (Lam.) Juss. ex Gagnep.	-	-	KF544861
<i>Tetrastigma annamense</i> Gagnep.	V9777	Vietnam	MN852313
<i>Tetrastigma fruticosum</i> Soulad., Phonp. & Tagane	Z534 (FOF, KAG, VNM)	Laos	PP920557
<i>Tetrastigma campylocarpum</i> (Kurz) Planch.	-	-	KF544880
<i>Tetrastigma harmandii</i> Planch.	<i>J.F. Barcelona & P.B. Pelser 4142</i> (CANU/PNH)	Miag-ao Iloilo Prov., Philippines	KT597213
<i>Tetrastigma hemsleyanum</i> Diels & Gilg	-	China	MW296875
<i>Tetrastigma henryi</i> Gagnep.	<i>DH 55</i> (PE)	China	MW408529
<i>Tetrastigma lanyuense</i> C.E.Chang	<i>Wen 9404</i> (US)	Taiwan	KT344625
<i>Tetrastigma obtectum</i> Gagnep.	Nie Meng (KUN, US)	China	KT344626
<i>Tetrastigma planicaule</i> (Hook.f.) Gagnep.	<i>DH 54</i> (PE)	China	MW408528
<i>Tetrastigma papillosum</i> (Blume) Planch.	<i>J.F. Barcelona & P.B. Pelser 3778</i> (No voucher)	Philippines	KT597170
<i>Tetrastigma philippinense</i> Merr.	<i>Obico 904</i> (CANU/PUH)	Philippines	MT522957
<i>Tetrastigma sulcatum</i> (M.A.Lawson) Gamble	<i>Tamhankar & More s.n.</i>	India	KF544883
<i>Tetrastigma trifoliolatum</i> Merr.	<i>Obico 983</i> (CANU/PUH)	Philippines	MT522958