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Untangling a vine and its parasite: Host specificity of Philippine *Rafflesia* (Rafflesiaceae)

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■ APPENDIX S1. NOTES ON *TETRASTIGMA* IDENTIFICATIONS AND TAXONOMY

As outlined in the main text of this paper, the identifications of our *Tetrastigma* (Miq.) Planch. specimens are provisional. This is in part due to the absence of reproductive structures in most of our specimens, but mostly because a taxonomic revision of *Tetrastigma* is still wanting. With the aim of providing information for future studies involving Philippine *Tetrastigma*, we have here provided some notes about each of the eight *Tetrastigma* lineages that we encountered during our fieldwork. These lineages are illustrated with photographs that are available from the PhytoImages website (Nickrent & al., 2006). A checklist of Philippine *Tetrastigma* species can be found on the Co's Digital Flora of the Philippines website (Pelsner & al., 2011–).

***Tetrastigma ellipticum* Merr. s.l.** — The plants that we refer to by this name can be recognized by the presence of forked tendrils and palmately compound leaves with 1, 3, or 5 leaflets. They form a clade with three Philippine specimens that were identified as *T. laxum* Merr. by Chen & al. (2011; *Wen* 8267!, 8278!, and 8314!, as *T. ellipticum* in US), a Philippine specimen included in their study as *T. ellipticum* (*Wen* 8260, as *T. glabratum* (Blume) Planch. in US), and two unidentified *Tetrastigma* plants from Indonesia (Chen & al., 2011; Lu & al., 2013).

According to Merrill (1916), *T. ellipticum* and *T. laxum* are morphologically similar to each other and a third species: *T. brunneum* Merr. All three species have palmately compound leaves (Merrill, 1912a, 1916). Those of *T. brunneum* have three or five leaflets, whereas the leaves of *T. ellipticum* and *T. laxum* are trifoliolate (Merrill, 1912a, 1916). Merrill (1916) wrote that *T. laxum* has “entirely different male flowers” (p. 141) than *T. brunneum* has, but did not observe the flowers of *T. ellipticum*. He also noted that *T. ellipticum* differs from *T. laxum* in having larger leaflets (Merrill, 1916). Merrill did not describe the tendrils of *T. ellipticum* and *T. laxum*, but wrote that those of *T. brunneum* are forked (Merrill, 1912a).

Because they have forked tendrils and palmately compound leaves, the specimens included in the study of Chen & al. (2011) as *T. laxum* indeed match this alliance of three *Tetrastigma* species well if we assume that *T. ellipticum* and *T. laxum* do not strictly have trifoliolate leaves (Merrill, 1916), but that these can also have five leaflets. Unfortunately, we were not able to view the voucher specimen of their *T. ellipticum* sequences, because it could not be located in US. Chen & al. (2011) also included a Philippine specimen identified as *T. brunneum* (*Wen* 8240), in their studies. Interestingly, however, it is only distantly related to their *T. ellipticum* and *T. laxum* accessions. Also this specimen could not be located in US.

Some of our specimens appear to be most closely related to Chen & al.'s (2011) *T. laxum* accessions and others form a clade with their accession of *T. ellipticum*. These clades, however, do not show the species-diagnostic differences in leaflet size that Merrill (1916) referred to. In fact, leaflet size was quite variable in both clades and exceeded the range of measurements provided by Merrill (1916) for both species. This might suggest that these species names may need to be synonymized or that

other differences between *T. ellipticum* and *T. laxum* remain to be discovered. Because of the morphological resemblance between our specimens and *T. brunneum*, we feel that future studies are also needed to confirm that *T. brunneum* is indeed only distantly related to *T. ellipticum* and *T. laxum*, as suggested by the findings of Chen & al. (2011).

Because we are confident that the phylogenetic affinities of our specimens lie with *T. ellipticum* and *T. laxum* (and perhaps *T. brunneum*), but cannot determine if they represent either of these species or both, we refer to them as members of the *T. ellipticum* s.l. lineage in this paper.

***Tetrastigma* aff. *glabratum* (Blume) Planch.** — Several specimens from Mindanao form a clade with an unidentified specimen from Luzon that was included in the study of Chen & al. (2011; *Wen* 8256; Fig. 1), but could not be located in US. This clade is, in turn, placed in a polytomy with a clade composed of specimens that were identified as *T. glabratum* and *T. cf. tuberculatum* (Blume) Latiff from Indonesia and *T. lawsonii* (King) Burkill from Singapore.

Tetrastigma tuberculatum (Blume) Latiff is a superfluous name and synonym of *T. coriaceum* (DC.) Gagnep. (Veldkamp, 2008, 2009). It is, however, likely that Chen & al.'s (2011) *T. cf. tuberculatum* specimen belongs to a different species than *T. coriaceum*, because their other accessions of *T. coriaceum* (as *T. tuberculatum*) and those of a specimen of *T. coriaceum* that we sequenced for this study are phylogenetically distant (Fig. 1). Their *T. cf. tuberculatum* specimen (*Wen* 10280) is filed as *T. lanceolarium* (Roxb.) Planch. in US. This name has been often misapplied to plants now recognized as *T. coriaceum* (Veldkamp, 2008, 2009). We have not been able to access *Wen* 10280 and it is not clear to us which species it represents. *Tetrastigma coriaceum* has been reported as the host species of *Rafflesia lobata* (Galang & Madulid, 2006; Galang, 2009) and *R. mira* (Madulid & al., 2006) as *T. tuberculatum*. We were not able to verify these host identifications, because voucher specimens could not be located. We did not observe *T. coriaceum* as a host species in the *Rafflesia* populations from which it was reported. In addition, despite extensive sampling throughout the Philippines, we never encountered *T. coriaceum* and can therefore not confirm its presence in the country.

Also the name *T. lawsonii* has a very complex history (Veldkamp, 2008). It is an additional superfluous name and synonym of *T. coriaceum*. To complicate matters further, the name *T. lawsonii* has been misapplied to *T. latiffii* Veldk. (Veldkamp, 2008). The two specimens identified as *T. lawsonii* (*Wen* 7503 and *Wen* 7505, US) by Chen & al. (2011) were not available to us and we don't know which species they represent.

Tetrastigma glabratum has been tentatively reported for the Philippines (Robinson, 1911; Merrill, 1923). This species has pedate leaves that have three or five leaflets and unforked tendrils. Although our specimens indeed have unforked tendrils, they typically have leaves with five leaflets and only occasionally four, six, or seven. In addition, according to Planchon (1887), the leaf venation of *T. glabratum* is inconspicuous, but our specimens stand out among other Philippine *Tetrastigma* lineages in having quite prominently sunken secondary veins

(Table 1). Our specimens might therefore represent a different species.

***Tetrastigma harmandii* Planch.** — *Tetrastigma harmandii* can be recognized by the combination of pedately compound leaves that usually have five leaflets, glossy dark green leaflets with very faint secondary nerves, unforked tendrils, a rather compact tomentose inflorescence, and light brown fruits (Planchon, 1887; Merrill, 1912b, 1916). Our specimens match this species very well and, as reported for *T. harmandii*, are used as a souring agent in cooking (Merrill, 1916; Brown, 1920). Shoots with only trifoliolate leaves and without reproductive parts can easily be confused with *T. loheri* Gagnep. s.l. (see below), because also some specimens of that species have quite glossy leaves without prominent secondary veins.

Yahya & al. (2010) reported *T. harmandii* as the single host plant of *Rafflesia lagascae* (as *R. manillana*) on Mt. Makiling. We could not verify this identification, because vouchers were not preserved, and did not find this species as a host plant of *R. lagascae* on Mt. Makiling or elsewhere in the Philippines.

***Tetrastigma loheri* Gagnep. s.l.** — Our specimens of this assemblage represent one of the most commonly encountered and collected *Tetrastigma* lineages in the Philippines. In our phylogenetic analyses, they form a clade with Chen & al.'s (2011) accessions of *T. strumarum* (Planch.) Gagnep. (*Wen 10757*, Indonesia; filed in US as *Cissus elongata* Roxb.), an unidentified plant (*Wen 10768*, Indonesia), and *T. diepenhorstii* (Miq.) Latiff (*Wen 8261*, Philippines). The accession of the latter specimen is found deeply nested among the Philippine specimens that we collected.

Wen & al. (2013) list the name *T. strumarum* as a synonym of *T. pachyphyllum* (Hemsl.) Chun in their account of Philippine *Tetrastigma*. Because the two accessions that were included under the name *T. pachyphyllum* (*Wen 10919*, Vietnam; *Wen 8319*, Philippines, filed as *T. trifoliolatum* Merr. in US) in the studies by Chen & al. (2011) are only distantly related to their *T. strumarum* accession, this either suggests that both names should not be synonymized or that one or more of these specimens has been misidentified. Another complicating factor concerning the use of the name *T. strumarum* for Philippine plants is that this name has been misapplied in the Philippines to *T. harmandii* plants (Merrill, 1916, 1923). Other than Wen & al. (2013), who did not cite any specimens or discuss this matter, we could not find any published account that lists “true” *T. pachyphyllum* or *T. strumarum* for the Philippines. The Philippines is also not included in the distribution area of *T. pachyphyllum* (with *T. strumarum* listed as a synonym) by Ren & Wen (2007). It is therefore unclear if *T. pachyphyllum* or *T. strumarum* occur in the Philippines. Because we were not able to access the specimen included by Chen & al. (2011) as *T. strumarum*, we cannot verify its identity or determine if it is conspecific with the Philippine specimens that were collected for our study.

According to Latiff (2001), *T. diepenhorstii* and *T. trifoliolatum* are synonymous. Chen & al. (2011), however, noticed that their accessions under both names are phylogenetically distant. Although this could indicate that these names represent distinct species, it is possible that specimens representing

them in Chen & al. (2011) have been misidentified. The voucher specimen of the *T. diepenhorstii* accession used by Chen & al. (2011) in their phylogenetic analyses (*Wen 8261*) could not be located by US staff and we can therefore not determine if it is morphologically similar to our specimens, but considering that it is deeply nested among them, this is likely.

Tetrastigma diepenhorstii and *T. trifoliolatum* are morphologically similar to *T. loheri* (incl. syn. *T. philippinense* Merr., Merrill 1923). They share consistently trifoliolate leaves and a relatively compact infructescence (Miquel, 1860; Gagnepain, 1910; Merrill, 1912a, 1914; Latiff 2001). In vegetative morphology, *T. diepenhorstii* (incl. *T. trifoliolatum*) differs from *T. loheri* in a longer petiole (ca. 12 cm vs. 1–2.5 cm) and petiolule of the terminal leaflet (8 cm vs. 1–2 cm; Merrill, 1912a, 1914). The specimens included in our study are very variable in the size, shape, length/width ratio, margin, and venation of their leaflets, and in the length of their petioles and petiolules, but we did not observe any phylogenetic structuring of these characters in our DNA sequence phylogenies.

Taxonomic descriptions, including those in the protologues (Miquel, 1860; Merrill, 1914), of *T. diepenhorstii* and *T. trifoliolatum* are not very detailed. Philippine plants of *T. loheri* (as *T. philippinense*) have been described in more detail (Merrill, 1912a). Merrill's protologue of *T. philippinense* mentions unforked tendrils, trifoliolate leaves, and oblong-obovoid fruits that first turn red and subsequently dark-purple or black (Merrill, 1912a). These characters have also been observed in our specimens, although one specimen displays a forked tendril (*Barcelona & al. 4007*) and another has unifoliolate leaves in addition to trifoliolate leaves (*Barcelona & al. 4105*). Only very few flowering specimens were observed, but their inflorescence and flower morphology is in accordance with the information presented in the protologue of *T. philippinense*. Because of the close morphological similarity of our plants with *T. loheri* (as *T. philippinense*), the uncertain taxonomic status of *T. diepenhorstii*, *T. loheri*, and *T. trifoliolatum*, and the large diversity in vegetative morphology observed among our specimens, we refer to these specimens as the *T. loheri* s.l. lineage. More detailed taxonomic studies are needed to determine if this alliance is best considered a single species or if it needs a more narrow delimitation.

Chen & al. (2011) included sequences of a specimen identified as *T. loheri* (*Wen 10202*, Indonesia; filed in US as *T. dubium* (M.A. Lawson) Planch.) in their analyses. This accession is phylogenetically distant from the *T. loheri* s.l. lineage. Because the voucher specimen of this accession could not be located in US, we cannot verify its identification.

Fernando & Ong (2005) listed *T. loheri* as a host plant of *R. mira*. The voucher specimen of this plant can, however, not be located in LBC and despite sampling most of the known host plants of *R. mira*, we only identified *T. cf. magnum* as a host.

***Tetrastigma cf. magnum* Merr.** — Several *Tetrastigma* specimens from various parts of the Philippines form a clade that is sister to Chinese and Thai plants that were identified as *T. cruciatum* Craib & Gagnep. by Chen & al. (2011; Fig. 1). Our specimens are characterized by pedate leaves that have

five or seven leaflets, an unforked tendril, white fruits that are globose or wider than long, and relatively open infructescences (Table 1). In addition, the members of this clade typically have relatively large leaves with at least some leaflets longer than 14 cm. Among the *Tetrastigma* species reported for the Philippines, they most closely resemble *T. everettii* Merr. and *T. magnum*. These two species are similar to each other leaf morphology and size, although *T. everetti* is reported to have fewer secondary veins per leaflet (6–10 per side) than *T. magnum* (12–15) and the petioles of *T. everetti* are described to be longer (13–16 cm) than those of *T. magnum* (8–12 cm; Merrill, 1916). In addition, *T. everettii* has a pubescent inflorescence, whereas that of *T. magnum* is glabrous (Merrill, 1916). The leaflets of our specimens have fewer secondary veins (5–11) than what is listed for *T. magnum* in its protologue (12–15; Merrill, 1916), although the leaflets of the three isotypes that we have seen (*Ramos 13610*; K, L, US) only have 6–9 secondary veins per side. The length of the petioles of our specimens varies between 4 and 20 cm and they therefore span the size ranges that have been reported for both species. We have not seen any flowering specimens and therefore could not determine if the inflorescence is pubescent. If so, this indumentum is caducous, because the infructescences of our specimens are glabrous. Merrill (1916) described the fruits of *T. magnum* as obovoid, but those of our specimens are globose or wider than long. He did not observe fruits of *T. everettii*.

***Tetrastigma papillosum* (Blume) Planch.** — Mature individuals of *T. papillosum* are easily recognized. They have internodes and petioles that are densely covered with conspicuous spine-like excrescences (Blume, 1825; Planchon, 1887; Latiff, 1983). These are absent in other Philippine *Tetrastigma* species. In addition, the leaves of *T. papillosum* are trifoliolate and the tendril is forked. Our specimens form a clade that is placed in a polytomy with, amongst others, a Malaysian specimen (*Wen 8401*; filed in US as *T. lawsonii*) that was identified as *T. papillosum* by Chen & al. (2011; Fig. 1). Chen & al.'s (2011) Philippine accession of *T. brunneum* (*Wen 8240*; not located in US) is also placed in this polytomy. This species resembles *T. papillosum* in having forked tendrils and can similarly have trifoliolate leaves. However, the leaves of *T. brunneum* are palmately compound. In contrast, those of *T. papillosum* appear to be pedately compound, because one of our specimens (*Barcelona & al. 3778*) has a single leaf with four leaflets in a pedate arrangement. *Tetrastigma brunneum* lacks the characteristic spine-like excrescences of *T. papillosum*.

Tetrastigma papillosum was reported as a host plant of *R. schadenbergiana* (Barcelona & al., 2009b), but further examination of the voucher specimen revealed that it is in fact *T. aff. glabratum*.

***Tetrastigma cf. scariosum* (Blume) Planch.** — Several Philippine specimens from Luzon, Visayas and Mindanao form a clade with specimens identified as *T. piscarpum* (Miq.) Planch. (*Wen 10185*, Indonesia; filed in US as *Tetrastigma* sp.) and *T. loheri* (*Wen 10202*, Indonesia; filed in US as *T. dubium*) in Chen & al. (2011; Fig. 1). The name *T. piscarpum* is considered a synonym of *T. scariosum* by Veldkamp

(2009) and we followed his conclusion in this paper. Of these two species, our specimens match *T. scariosum* best. They have leaves with usually five or seven leaflets that are pedately arranged, although some species also bear leaves with three leaflets. Jackes (1989) reported that the leaves of *T. scariosum* (as *T. piscarpum*) typically have nine and sometimes seven or eleven leaflets, although Blume (1825), Planchon (1887), and Latiff (1991; as *T. piscarpum*) mentioned leaves with five leaflets for this species and Miquel (1863) and Planchon (1887) (both as *T. piscarpum*) listed seven. *Tetrastigma scariosum* has forked tendrils (Jackes, 1989), and so do our specimens. Because none of our specimens are fertile and leaflet morphology is quite variable in *Tetrastigma*, a comparison with photos of the type specimens of *T. scariosum* and its various heterotypic synonyms (as detailed by Veldkamp, 2009) did not allow us to conclusively determine if our plants indeed represent this species. The isotypes of the synonym *T. godefroyanum* Planch., however, nicely show pedately compound leaves with predominantly seven leaflets and forked tendrils.

Tetrastigma loheri never has more than three leaflets per leaf and possesses an unbranched tendril (see discussion above). This suggests that *Wen 10202* might have been misidentified. Unfortunately, this specimen could not be located in US. Because the online label information states that it has leaves with three leaflets, it might be a fragment of a *T. scariosum* plant that bears trifoliolate leaves.

Tetrastigma scariosum has only recently been reported from the Philippines. Latiff annotated Philippine herbarium specimens as *T. piscarpum* in A and NY in 1985 and their morphology resembles that of our plants. It appears to have been first listed by Madulid & al. (2007) for the Philippines in the printed literature, although their record most likely refers to *T. cf. magnum* (see main text of this paper). The species is also included in an overview of Philippine Vitaceae by Wen & al. (2013; as *T. piscarpum*), although without further discussion.

Interestingly, *T. scariosum* has been reported as a host of *Rafflesia patma* Blume in Indonesia (e.g., Blume, 1825; Veldkamp, 2009), but has not been identified as a host of Philippine *Rafflesia*.

***Tetrastigma* sp. A.** — This lineage is composed of specimens that form a clade with an accession of a Philippine specimen that was identified by Chen & al. (2011) as *T. pachyphyllum* (*Wen 8319*; filed in US as *T. trifoliolatum*). As outlined above (under *T. loheri* s.l.), it is unclear if *T. pachyphyllum* occurs in the Philippines. Furthermore, we were not able to access *Wen 8319* to determine if it is morphologically similar to our specimens.

Our specimens have pedate leaves that are occasionally unifoliolate, but much more commonly have three or five leaflets. They have unforked tendrils and the secondary venation is clear and sunken on the adaxial surface (Table 1). We did not observe flowering or fruiting plants. Some of our specimens with trifoliolate leaves are quite similar to *T. loheri* s.l. plants with clear and sunken secondary veins. It is presently unclear which *Tetrastigma* species our specimens represent.

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