

Spatial and temporal diversification of *Tetrastigma* (Vitaceae)

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ABSTRACT. The spatial and temporal diversification of *Tetrastigma* Planch., a genus of the grape family Vitaceae with a wide distribution throughout subtropical and tropical Asia to Australia, was examined through phylogenetic and biogeographic analyses. The times of divergence within *Tetrastigma* were estimated with the Bayesian approach based on sequence data from four plastid (*atpB-rbcL*, *rps16*, *trnL-F*, and *psbA-trnH*) markers using the computer program BEAST. The divergence time between *Tetrastigma* and its closely relative *Cayratia* was estimated as the early Eocene around 50.6 million years ago (mya), with 95% HPD: 36.3–65.3 mya. The age of the crown group of *Tetrastigma* was dated to be late Eocene (36.9 mya, with 95% HPD: 25.7–49.3 mya). Biogeographic analyses using LAGRANGE suggested that the Sino–Himalayan region (and the adjacent Indochina) was the most likely ancestral area for *Tetrastigma*. Most *Tetrastigma* species sampled from the Malesian region were nested within clades of the Sino–Himalayan and Indochina region. A few Malesian species primarily from SE Sulawesi, the Philippines and New Guinea are not associated with the Sino–Himalayan and Indochina species and formed separated clades. The results suggest that *Tetrastigma* species in the Malesian region have complex biogeographic origins and continental Asia served as an important source area for the Malesian members of the genus.

Keywords. BEAST, biogeography, dating, LAGRANGE, *Tetrastigma*, Vitaceae

Introduction

Tetrastigma Planch. is one of the 14 currently recognised genera of the grape family Vitaceae (Wen 2007). The genus contains approximately 95 species and has a wide distribution in tropical and subtropical Asia, extending to Australia, ranging from India to China, across SE Asia eastward to Fiji (Fig. 1; Chen et al. 2011). *Tetrastigma* is characterised by unbranched to digitately branched tendrils, a dioecious sexual system, and 4-lobed stigmas in female flowers (Wen 2007). *Tetrastigma* is well-known in Southeast (SE) Asia for being the host plants for all three genera of Rafflesiaceae, which contains the largest flower of the world (Meijer 1997, Barkman et al. 2004).



Fig. 1. The geographic distribution of *Tetrastigma* in Asia and Australia (gray area). “a–d” indicates four areas of endemism used in the LAGRANGE analysis: a, the Malesian region; b, Sino–Himalayan and Indochina region; c, Africa; and d, New World.

Recently the genus attracted more scientific attention with the reported horizontal gene transfer of mitochondrial genes (e.g., *nad1B-C* and *apt1*) from the *Tetrastigma* host to the parasite of *Rafflesia* R.Br. (Davis & Wurdack 2004, Barkman et al. 2007). Several species of the genus are widely cultivated as climbing ornamentals (Wen 2007). *Tetrastigma hemsleyanum* Diels & Gilg is an important Chinese folk medicine for treating hepatitis, fever, pneumonia, rheumatism, and sore throat (Liu et al. 2002).

Southeast Asia and the West Pacific have long attracted the attention of biogeographers. The waters of SE Asia contain the highest marine faunal diversity in the world (Briggs 1974; Paulay 1997). Biodiversity in this region shows several patterns of origin between two complicated palaeocontinents separated for a considerable period of time. Two main patterns have often been recognised: (1) a pattern of Southeast Asian elements (perhaps of Laurasian origin) and (2) a pattern of Australian elements (perhaps of Gondwanan origin). Some recent phylogenetic and biogeographic studies have shed important insights into the evolution of plant, insect, fish, and animal distribution patterns in this region (see Butlin et al. 1998; Marwick 2009; Clouse & Girbet 2010; Esselstyn & Oliveros 2010; Renner et al. 2010). Yet the Malesian flora comprises an estimated 42,000 plant species (Roos 1993), with only about 15% being revised during the last 50 years. Thus biogeographic analyses of more plant genera or lineages in this region are very much needed.

Several lines of evidence have shown that good dispersers such as some plants and insects from Eurasia can dominate the entire SE Asia and extend to the Pacific islands (Gressitt 1982, Baker et al. 1998). *Tetrastigma* is a good disperser, bearing berries and often dispersed by fruit-eating birds, bats and mammals (Tiffney &

Barghoorn 1976, Moran et al. 2009). The genus thus provides another opportunity to reconstruct the biogeographic diversification of plants in SE Asia and the West Pacific.

Tetrastigma is widely distributed in the Sino–Himalayan / Indochina region and the Malesian region. The Malesian region comprises west Malesia (the Sunda shelf: Malay Peninsula, Sumatra, Borneo, and West Java), central Malesia (Wallacea: part of Java, the Philippines, Sulawesi, the Lesser Sunda Islands, and the Moluccas), and east Malesia (the Sahul Shelf: New Guinea) as subunits (van Welzen et al. 2005). The Wallace's line separates the west and the east Malesian flora, the transition area between west and east Malesia is known as Wallacea, i.e., central Malesia. The tectonic history of the Malesian region is complicated because of the two waves of Australian slivers moving towards the Eurasian plate. The first wave formed SE Asia and the west part of the Malay Archipelago to the Wallace's or the Weber's line around 90 million years ago (mya). The second wave made SE Asia collide with the West Pacific in the middle Miocene (van Welzen et al. 2003). Besides this complicated tectonic history and cycles of glacials, the origins of plant taxa may also be complex. It is still poorly known which taxa in this region dispersed from the Sino-Himalayan region or from the West Pacific region. Because species of *Tetrastigma* occupy the entire Malesian region, it seems a good model to test the dispersal pathways in the Malesian region.

The fossil records of the Vitaceae in the Northern Hemisphere during the Tertiary are well documented. Pollen, fruit, seed and leaf fossils have been recorded from the late Paleocene to the Pleistocene (Collinson 1983, Cervallos-Ferriz & Stockey 1990, Taylor 1990, Wheeler & Lapasha 1994). Fossil seeds of Vitaceae are commonly found in many Tertiary beds in the Northern Hemisphere (Reid & Chandler 1933; Kirchheimer 1939; Miki 1956; Dorofeev 1957, 1963; Chandler 1925, 1957, 1961a, 1961b, 1962, 1963, 1964, 1978; Tiffney & Barghoorn 1976; Chen & Manchester 2007). Because the fossils resemble extant seeds, these fossil seeds have usually been identified to extant genera (Chen & Manchester 2007). Many of those fossils were designated to the genus *Vitis* and others were included in *Ampelopsis*, *Ampelocissus*, *Parthenocissus*; yet only a few fossils were reported to be *Tetrastigma* and *Cayratia* (Tiffney & Barghoorn 1976, Chen & Manchester 2007, Chen 2009). In general, fossil seeds of Vitaceae were relatively diverse in Europe (Chen 2009). About 13 fossil species of *Tetrastigma* have been reported from the early Eocene to Pliocene (Kirchheimer 1938; Miki 1956; Chandler 1925, 1961ab, 1962; Reid & Chandler 1933; Teodoridis 2003). Most of them varied morphologically and were from Europe in the early Tertiary and only two species (*T. japonica* Miki and *T. tazimiensis* Miki) were from Asia (Japan) from the Pliocene. Chen & Manchester (2007) carefully examined the fossils of *Ampelocissus* concerning seed morphological characters. They questioned the generic assignment of most *Tetrastigma* fossil seeds, which resemble those of *Ampelocissus* and *Ampelopsis*. They transferred two *Tetrastigma* fossils to *Ampelocissus*: with *T. lobatum* Chandler now being *Ampelocissus lobatum* (Chandler) Chen & Manchester, and *Tetrastigma chandleri* now as *Ampelocissus chandleri* (Kirchheimer) Chen & Manchester (Chen & Manchester 2007). There may be no reliable *Tetrastigma* fossil seeds so far (S. Manchester, pers. comm.). Moreover, the oldest fossil in Vitaceae was *Ampelocissus parvisemina* Chen & Manchester from

the late Paleocene of North Dakota and the early-middle Eocene of Oregon (Chen & Manchester 2007).

Chen et al. (2011) conducted a phylogenetic analysis of *Tetrastigma* using four plastid markers. Yet the biogeography of the genus has never been explored. The objectives of this study are to (1) estimate the divergence times of *Tetrastigma* clades, and (2) infer the biogeographic diversification history of the genus.

Materials and methods

Estimation of divergence times

Representatives from the entire grape family plus *Leea* were sampled to help date the ages of *Tetrastigma* and its relatives with both direct fossil and secondary calibrations in Vitales. Sequences used in the dating analysis are shown in Appendix A and were derived from a phylogenetic analysis of the genus by Chen et al. (2011). We used the Bayesian dating method based on a relaxed-clock model to estimate divergence times (Thorne et al. 1998; Thorne & Kishino 2002; Drummond et al. 2006). The Bayesian coalescent approach to estimate the times of each clade in *Tetrastigma* and their credibility intervals was implemented in the computer program BEAST version 1.4.7 (Drummond & Rambaut 2007), which employs a Bayesian Markov chain Monte Carlo (MCMC) to co-estimate topology, substitution rates and node ages. All analyses were performed using the GTR model of nucleotide substitution with a gamma and invariant sites distribution with four rate categories. The tree prior model (constant size) was implemented in the analysis, with rate variation across branches assumed to be uncorrelated and lognormally distributed (Drummond et al. 2006). The final estimates were obtained using the model that yielded the highest posterior probability. Posterior distributions of parameters were approximated using two independent MCMC analyses of 20,000,000 generations with 10% burn-in. Samples from the two chains which yielded similar results were combined and convergence of the chains was checked using the program Tracer 1.3 (Rambaut & Drummond 2004).

Ancestral area analyses

Reconstruction of ancestral areas on a phylogeny is important to understand the biogeographic diversification history of a lineage, as it permits the inference of the place of origin and dispersal routes of organisms. Dispersal-viceariance (DIVA; Ronquist 1996, 1997) analysis is often used to construct the biogeographic history. But for DIVA analyses the phylogeny of a group needs to be well resolved. The procedure used in the computer program LARANGE differs from the DIVA methods in that it allows a broader range of speciation models and also incorporates any available temporal information such as divergence times and dispersal opportunities. We thus employed the maximum likelihood-based method LAGRANGE (Ree et al. 2005, Ree & Smith 2008) to reconstruct the diversification of *Tetrastigma* and its close relatives. Four areas of endemism were defined according to their distribution: (a) the Malesian region, (b) the Sino–Himalayan and Indochina region, (c) Africa, and

(d) the New World. The maximum number of areas in ancestral ranges was set as two in LAGRANGE, as no species of Vitaceae is distributed in more than two areas of endemism.

Vitaceae fossil constraints

We constrained the ages of two nodes in the phylogeny of *Tetrastigma* and its close relatives in Vitaceae (Fig. 2). The stem lineage of *Ampelocissus* and *Vitis* was constrained to be 60 ± 5.0 mya old (node) in Fig. 2 based on fossil seeds of *Ampelocissus parvisemina* from the late Paleocene of North Dakota and the early to middle Eocene of Oregon (Chen & Manchester 2007). Wikström et al. (2001) reported the estimated divergence time between *Leea* and *Vitis* as 80–92 mya. Recently Magallón & Castillo (2009) suggested an older age from 90.65 (90.47–90.84) to 90.82 (90.64–91) mya for the origin of Vitaceae using different relaxed or constraint schemes, although there is no direct credible fossil evidence for Vitaceae or Leeaceae in the Cretaceous. It is curious that the distinctive seed morphology of this clade, readily observable in many Paleogene localities, is missing from well studied Cretaceous deposits. The inferences from Magallón & Castillo (2009) and Wikström et al. (2001) are close, although the latter were criticised for the nonparametric rate smoothing method and for calibrating their tree using only a single calibration point. Bell et al. (2010), however, suggested a time ranging from 65 (45–81) to 48 (21–79) Ma for the stem age of Vitaceae. Although their estimates were based on 36 fossil calibrations and a relaxed approach in dating the whole angiosperm groups, they seem to have underestimated the age for Vitaceae because the estimates were younger than the age suggested by fossil evidence (e.g., in Chen & Manchester 2007). We thus constrained the stem of Vitaceae to be 85 ± 5.0 mya (Fig. 2), a strategy similar to that of Nie et al. (2010). We did not use any *Tetrastigma* fossils from the Tertiary (Kirchheimer 1938; Miki 1956, Chandler 1925, 1961ab, 1962; Reid & Chandler, 1933; Teodoridis 2003), as Chen & Manchester (2007) questioned the placement of all these fossils in the genus.

Results

The chronogram of *Tetrastigma* and its relatives from Vitaceae based on combined plastid *atpB-rbcL*, *rps16*, *trnL-F*, and *psbA-trnH* data is shown in Fig. 2. The age of the *Tetrastigma* stem was estimated to be 50.6 mya (with a 95% highest posterior density [HPD] interval of 36.4–65.3 mya) in the early Tertiary. The crown age of *Tetrastigma* was estimated to be 36.94 mya (with a 95% HPD: 25.7–49.3 mya) approximately in the late Eocene. Node ages of eight major clades (clades A–H) are shown in Table 1. Clade B formed a well-supported clade with the large Clade A based on Bayesian analysis (Fig. 2). Three species (*T. glabratum* Wen10670, *T. lawsoni* Wen7505, *T. tuberculatum* Wen10280) in Clade B were collected from Java, Singapore, and SE Sulawesi. The crown age of Clade D was estimated to be 7.8 mya (95% HPD: 2.2–14.5 mya, node D in Fig. 2) including two endemic species of the Philippines (*T. ellipticum*, and *T. laxum*) and a new undescribed species collected from SE Sulawesi. The small Clade

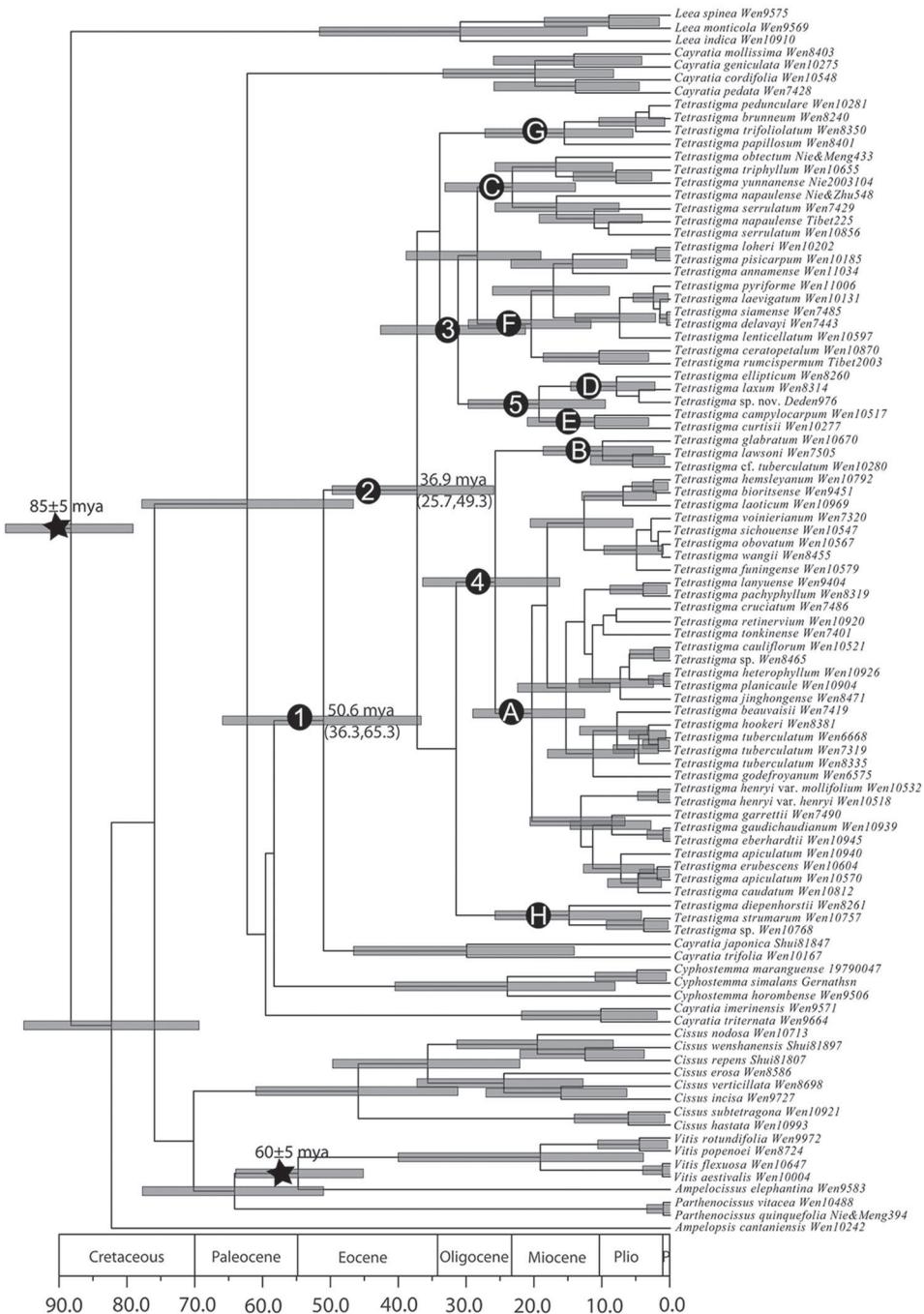


Fig. 2. Chronogram of *Tetastigma* and its relatives from Vitaceae based on combined plastid data (*atpB-rbcL*, *rps16*, *trnL-F*, and *psbA-trnH*) inferred from BEAST. Gray bars represent the 95% high posterior density credibility interval for node ages. Calibration points are indicated with black asterisks.

E was sister to Clade D with good Bayesian posterior probability support (node 5 in Fig. 2). Clade D only contained central Malesian species of *Tetrastigma*. However, the sister Clade E contained two biogeographically disjunct species, *T. campylocarpum* Planch. distributed from India to the Sino–Himalayan and Indochina region and *T. curtisii* (Ridl.) Suess. & Suess. of the Malesian region. The crown age of Clade E was estimated to be 11.0 mya (95% HPD: 3.1–20.8 mya, node E in Fig. 2). The ancestral area of Clade E was inferred to be widespread in the Sino–Himalayan and Indochina region and the Malesian region (node E in Fig. 3). Clade F included seven species from the Sino–Himalayan and Indochina region and three species from the Malesian region (*T. loheri* Wen 10202 and *T. piscicarpum* Wen 10185 from SE Sulawesi and *T. laevigatum* Wen 10131 from West Java). The east and central Malesian *T. diepenhorstii* Wen 10812 and west Malesian endemic *T. strumarum* Wen 10757 and *T. sp.* Wen10768 constituted Clade H (Fig. 3). The crown age of Clade H was estimated to be 14.8 mya (95% HPD: 4.2–25.6 mya, node H in Fig. 2) in the middle Miocene.

Reconstruction of ancestral areas with LAGRANGE suggested an ancestral distribution and early diversification of *Tetrastigma* in the Sino–Himalayan and Indochina region in Fig. 3. Subsequently, *Tetrastigma* species were dispersed from continental Asia to the Malesian region. The colonisation of the Sino–Himalayan and Indochina region occurred at nodes A and C and the colonisations of the Malesian region were supported by several nodes: B, D, G, and H (Fig. 3). The most widespread ancestral range appeared at nodes E and F (Fig. 3).

Table 1. Prior probability and posterior distribution estimates for *Tetrastigma* within the phylogenetic framework of Vitaceae. Mean dates were used as the divergence time of the nodes.

Node constrained and estimated	Posterior distribution Mean (mya)	95%HPD (mya)
Node 1	50.6	36.3–65.3
Node 2	36.9	25.7–49.3
Node 3	31.0	21.1–42.2
Node 4	25.5	16.1–36.1
Node 5	19.1	9.5–29.5
Node A	20.2	12.5–28.8
Node B	9.9	2.5–18.5
Node C	23.0	13.8–32.8
Node D	7.8	2.2–14.5
Node E	11.0	9.4–29.5
Node F	20.3	11.6–29.5
Node G	15.4	5.4–27.0
Node H	14.8	4.2–25.6

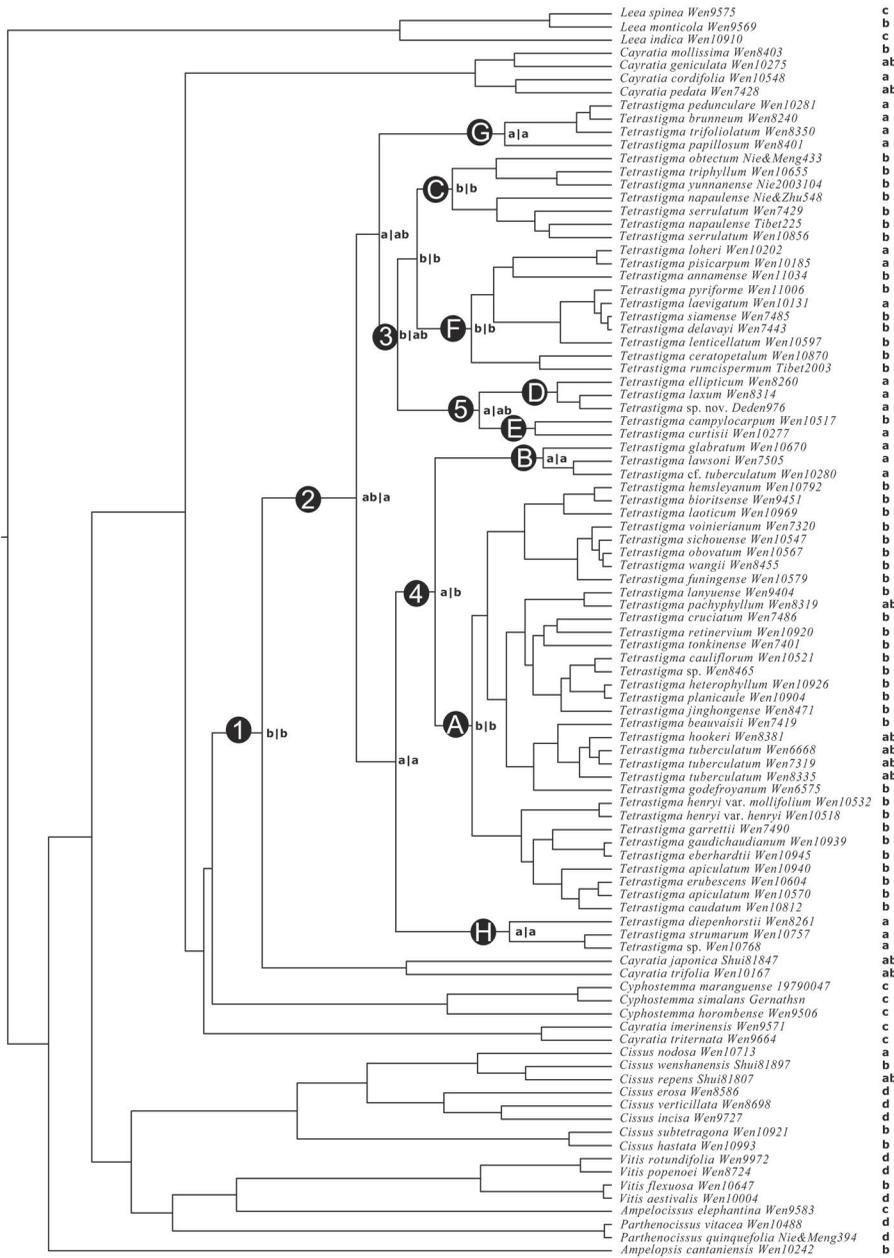


Fig. 3. Results of the LAGRANGE analysis of *Tetrastigma* and its close relatives. The tree was based on a 50% majority-rule consensus tree of a Bayesian Markov chain Monte Carlo (MCMC) analysis of the combined plastid data set. Estimated ancestral areas of the clades (Clades A–H) within *Tetrastigma* are shown on the nodes using circles (see details in the Discussion). The four areas of endemism are: a, the Malesian region; b, Sino–Himalayan and Indochina region; c, Africa; and d, New World. A slash in the result of LAGRANGE indicates the split of areas into two daughter lineages, i.e., left/right, where “up” and “down” are the ranges inherited by each descendant branch.

Discussion

Ancestral area and divergence times of Tetrastigma

Tetrastigma is distinctive in comparison with other genera of Vitaceae in its four-lobed stigma in female flowers and dioecious sexual system. Our phylogenetic analyses have suggested that *Tetrastigma* is nested within the genus *Cayratia* Juss. (also see Chen et al. 2011). *Tetrastigma* was estimated to have diverged from its closest relative in the early Eocene (50.6 mya, 95% HPD: 36.3–65.3 mya, node 1 in Fig. 2). The genus *Cayratia* is widely distributed in the Old World from Africa throughout Asia to Oceania. *Cayratia* has been shown to be paraphyletic (Rossetto et al. 2001, 2002, 2007; Ingrouille et al. 2002; Soejima & Wen 2006; Wen et al. 2007; Ren et al. 2011). Our analyses has divided the genus into three geographic clades: the African clade, and two clades in temperate to tropical Asia extending to Australia. *Tetrastigma* is sister to the clade including *Cayratia japonica* (Thunb.) Gagnep. and *C. trifolia* (L.) Domin, largely from temperate to tropical Asia. Thus, *Tetrastigma* may have originated in the Sino–Himalayan and Indochina region in the early Eocene.

The stem group of *Tetrastigma* is estimated to be 50.6 mya (95% HPD: 36.3–65.3 mya, node 1 in Fig. 2) approximately in the early Eocene. The crown of extant *Tetrastigma* is estimated to be 36.9 mya (95% HPD: 25.7–49.3 mya, node 2 in Fig. 2). The divergence of *Tetrastigma* species between the Sino–Himalayan and Indochina region and the Malesian region may have begun in the late Eocene. In our Bayesian analyses, at the beginning of *Tetrastigma* diversity, there are two Malesian clades (clades G and H in Fig. 2), and the Sino–Himalayan and Indochina and Malesian clades (clades A–F in Fig. 2). Improved understanding of SE Asia's physical history (Hall 2001, 2002; Metcalfe 2001; van Welzen 2005) suggests that only west Malesia (Malaysia, Sumatra, Borneo, Java, part of Sulawesi) was above water before the early Eocene. By the Miocene, the other islands (such as part of Sulawesi, Moluccas, part of the Lesser Sunda islands, and New Guinea) in the Malesian region got into their approximate present-day positions. With *Tetrastigma* fruits being dispersable berries, the migrations of *Tetrastigma* between the Sino–Himalayan and Indochina region and the Malesian region could have occurred at least three times, according to our results.

Biogeographic evolution of Tetrastigma in the Malesian region

Tetrastigma species from the Malesian region are scattered in six major clades (clades B, D, E, F, G, and H in Fig. 2). Some of the west and central Malesian *Tetrastigma* samples are nested within the clades of Sino–Himalayan and Indochina region (clades B, E, and F in Fig. 3). Thus, migrations of *Tetrastigma* species between the Sino–Himalayan and Indochina region and west Malesia may have occurred at least three times from the late Oligocene to the middle Miocene (Fig. 2). During these periods with climatic and sea level changes, species of *Tetrastigma* seem to have dispersed from the Sino–Himalayan / Indochina region to the Malesian region and also from the Malesian region back to the Sino–Himalayan / Indochina region.

Three species (*T. glabratum* Wen 10670, *T. lawsoni* Wen 7505, *T. tuberculatum* Wen 10280) in Clade B are widespread in Indochina and both west and central

Malesia. The divergence time between these two disjunctive clades is estimated to be 25.5 mya (95% HPD: 16.1–36.1 mya, node 4 in Fig. 2) in the late Oligocene and early Miocene. Throughout the Oligocene, a land connection between Borneo and Indochina was hypothesised to have existed (Pupilli 1973, Lloyd 1978). *Tetrastigma* species of the Sino-Himalayan and Indochina region may have dispersed into west and central Malesia through the land bridge or via direct long-distance dispersal. Furthermore, Clade F included seven species from the Sino-Himalayan and Indochina region as well as species from west and central Malesia (*T. loheri* Wen 10202 and *T. piscarpum* Wen 10185 from SE Sulawesi and *T. laevigatum* Wen 10131 from West Java). Although the phylogeny did not well resolve the relationships within Clade F, the clade apparently represents another biogeographic connection between continental Asia and the Malesian region. The Oligocene and earliest Miocene were periods with much drier and cooler climates; a major climatic change occurred in the early Miocene (Morley & Flenley 1987), a period with markedly warm and moist climatic conditions through a large part of SE Asia and East Asia (also see Morley 1998). During this period, the ancestor of Clade A may have diversified rapidly in the Sino-Himalayan / Indochina region, with 50% of *Tetrastigma* species now endemic to this region (see Li 1998; Chen et al. 2011).

Dispersal of *Tetrastigma* ancestors between west and central Malesian region and the Sino-Himalayan / Indochina region may also be shown in Clade G and node 3 (Fig. 3). In Clade G, the basally branched taxon *T. papillosum* (Bl.) Planch. is widely distributed from southwestern China, Thailand, Southeast Asia to New Guinea. The other three species in this clade are distributed in three areas of Malesia. Species in Clade D included two endemic species of the Philippines (*T. ellipticum* and *T. laxum*) and a new species collected from SE Sulawesi. The clade thus showed a close biogeographic connection between the Philippines and Sulawesi. During the Neogene, a volcanic arc existed along the north arm of Sulawesi and possible island chain connections may have existed to the Philippines (Moss & Wilson 1998). Moreover, many authors (Duffels 1990, Musser 1987, Balgooy 1987) have noted that the flora and fauna of the south arm of Sulawesi are different from the rest of Sulawesi, which may be explained by the geologic evidence that during much of the Tertiary, South Sulawesi was below sea level. But the rifting and rotating of South Sulawesi (including SE Sulawesi at present) and its accretion to North Sulawesi just occurred 10 mya ago (Hall 1998). With a global warm phase during the middle Miocene (Fulthorpe & Schlanger 1989), it is possible for migration through those island chains and connection lands between the Philippines and Sulawesi, i.e., west Malesia and central Malesia. Species endemic to central Malesia formed Clade D, sister to Clade E (Fig. 3) and nested with the Sino-Himalayan and Indochina region species. The ancestral area of Clade E is inferred to be the Sino-Himalayan and Indochina region and Malesian region (node E in Fig. 3). The crown age of Clade E is estimated to be 11.0 mya (95% HPD: 3.1–20.8 mya, node E in Fig. 2) in the middle Miocene. Two species are included in Clade E (Fig. 3), *T. campylocarpum* Planch. widely distributed from India to the Sino-Himalayan / Indochina region and *T. curtisii* (Ridl.) Suess. & Suess. occurring in west and central Malesia. Thus, the possible dispersal route of *Tetrastigma* species is most likely from

the Sino–Himalayan/Indochina region to west Malesia in the early Oligocene (node 3 in Fig. 2) and then to central Malesia after the middle Miocene (node 5 in Fig. 2).

Clade H contains the east Malesian and Papuan species of *Tetrastigma* (Fig. 3). The basally branching species of Clade H is *T. deipenhorstii* (Miq.) Latiff, a widespread species in west Malesia. It is probable that the east Malesian species dispersed from west Malesia. Our results are consistent with routes from the Sino–Himalayan / Indochina region to west Malesia and from west to central Malesia.

We need to test our hypotheses of *Tetrastigma* biogeography with additional sampling in both the Sino-Himalayan / Indochina region and the Malesian region and with improved resolution of the phylogeny and finer division of the areas of endemism. Nevertheless, our initial results add another case study on the complex biogeographic origins of Malesian plant taxa, and support the idea that continental Asia served as an important source area for the Malesian members of the genus.

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Appendix A. Taxa and accessions used for dating and biogeographic analysis of *Tetrastigma* and the outgroup taxa of Vitaceae and Leeaceae with their GenBank numbers. “–” indicates missing data. All voucher specimens are deposited at the US National Herbarium (US).

Species	Collection localities (and vouchers)	GenBank accessions <i>atpB-rbcL</i> , <i>rps16</i> , <i>trnL-F</i> , <i>psbA-trnH</i>
<i>Ampelocissus elephantina</i> Planch.	Madagascar, Antsiranana (<i>J. Wen</i> 9583)	HM585516, HM585792, HM585932, HM585659
<i>Ampelopsis cantoniensis</i> Planch.	Indonesia, SE Sulawesi (<i>J. Wen</i> 10242)	HM585517, HM585793, HM585933, HM585660
<i>Cayratia cordifolia</i> C.Y. Wu ex C.L. Li	China, Yunnan (<i>J. Wen</i> 10548)	HM585518, HM585794, HM585934, HM585661
<i>Cayratia geniculata</i> Gagnep.	Indonesia, SE Sulawesi (<i>J. Wen</i> 10275)	HM585519, HM585795, HM585935, HM585662
<i>Cayratia imerinensis</i> (Baker) Desc.	Madagascar, Antsiranana (<i>J. Wen</i> 9571)	HM585520, HM585796, HM585936, HM585663
<i>Cayratia japonica</i> (Thunb.) Gagnep.	China, Yunnan (<i>Y.-M.</i> <i>Shui</i> 81836)	HM585521, HM585797, HM585937, HM585664
<i>Cayratia mollissima</i> Gagnep.	Malaysia, Pahang (<i>J.</i> <i>Wen</i> 8403)	HM585522, HM585798, HM585938, HM585665
<i>Cayratia pedata</i> Gagnep.	Thailand, Chiang Mai (<i>J. Wen</i> 7428)	HM585523, HM585799, HM585939, –
<i>Cayratia trifolia</i> (L.) Domin	Indonesia, SE Sulawesi (<i>J. Wen</i> 10167)	HM585524, HM585800, HM585940, HM585666
<i>Cayratia triternata</i> (Baker) Desc.	Madagascar, Antsiranana (<i>J. Wen</i> 9664)	HM585525, HM585801, HM585941, HM585667
<i>Cissus erosa</i> Rich.	Peru, Arequipa (<i>J. Wen</i> 8586)	HM585526, HM585802, HM585942, HM585668
<i>Cissus hastata</i> Planch.	Vietnam, Danang (<i>J.</i> <i>Wen</i> 10993)	HM585527, HM585803, HM585943, HM585669
<i>Cissus incisa</i> Des Moul.	USA, Texas (<i>J. Wen</i> 9727)	HM585528, HM585804, HM585944, HM585670
<i>Cissus nodosa</i> Blume	Indonesia, Papua (<i>J.</i> <i>Wen</i> 10713)	HM585529, HM585805, HM585945, HM585671
<i>Cissus repens</i> Lam.	China, Yunnan (<i>Y.-M.</i> <i>Shui</i> 81807)	HM585530, HM585806, HM585946, HM585672
<i>Cissus subtetragona</i> Planch.	Vietnam, Ninh Binh (<i>J.</i> <i>Wen</i> 10921)	HM585531, HM585807, HM585947, HM585673
<i>Cissus verticillata</i> (L.) Nicolson & C.E. Jarvis	Mexico, Chiapas (<i>J.</i> <i>Wen</i> 8698)	HM585532, HM585808, HM585948, HM585674

<i>Cissus wenshanensis</i> C.L. Li	China, Yunnan (<i>Y.-M. Shui 81897</i>)	HM585533, HM585809, HM585949, HM585675
<i>Cyphostemma horombense</i> Desc.	Madagascar, Fianarantsoa (<i>J. Wen 9506</i>)	HM585534, HM585810, HM585950, HM585676
<i>Cyphostemma maranguense</i> (Gilg) Desc.	National Botanic Garden of Belgium (cult.) (<i>19790047</i>)	HM585535, HM585811, HM585951, HM585677
<i>Cyphostemma simulans</i> (C.A.Sm.) Wild & R.B. Drumm.	USA, Iowa (cult.) (<i>J. Gerrath s.n.</i>)	HM585536, HM585812, HM585952, HM585678
<i>Leea indica</i> (Burm.f.) Merr.	Vietnam, Ninh Binh (<i>J. Wen 10910</i>)	HM585537, HM585813, HM585953, HM585679
<i>Leea monticola</i> J. Wen	Madagascar, Antsiranana (<i>J. Wen 9569</i>)	HM585538, HM585814, HM585954, –
<i>Leea spinea</i> Desc.	Madagascar, Antsiranana (<i>J. Wen 9575</i>)	HM585539, HM585815, HM585955, –
<i>Parthenocissus vitacea</i> (Knerr) Hitchc.	China, Yunnan (cult.) (<i>Z.-L. Nie & Y. Meng 394</i>)	HM585540, HM585816, HM585956, HM585680
<i>Parthenocissus vitacea</i> (Knerr) Hitchc.	Canada, Quebec (<i>J. Wen 10488</i>)	HM585541, HM585817, HM585957, HM585681
<i>Tetrastigma annamense</i> Gagnep.	Vietnam, Lam Dong (<i>J. Wen 11034</i>)	HM585543, HM585819, HM585959, HM585683
<i>Tetrastigma apiculatum</i> Gagnep.	Vietnam, Hoa Binh (<i>J. Wen 10940</i>)	HM585546, HM585822, HM585962, HM585686
<i>Tetrastigma apiculatum</i> Gagnep.	China, Yunnan (<i>J. Wen 10570</i>)	HM585544, HM585820, HM585960, HM585684
<i>Tetrastigma beauvaisii</i> Gagnep.	Thailand, Mae Hong Son (<i>J. Wen 7419</i>)	HM585547, HM585823, HM585963, HM585687
<i>Tetrastigma bioritsense</i> (Hayata) Hsu & Kuoh	China, Taiwan (<i>J. Wen 9451</i>)	HM585548, HM585824, HM585964, HM585688
<i>Tetrastigma brunneum</i> Merr.	Philippines, Luzon (<i>J. Wen 8240</i>)	HM585549, HM585825, HM585965, HM585689
<i>Tetrastigma campylocarpum</i> Planch.	China, Yunnan (<i>J. Wen 10521</i>)	HM585552, HM585828, HM585968, HM585692
<i>Tetrastigma caudatum</i> Merr. & Chun	Vietnam, Vinh Phuc (<i>J. Wen 10812</i>)	HM585551, HM585827, HM585967, HM585691
<i>Tetrastigma cauliflorum</i> Merr.	China, Yunnan (<i>J. Wen 10521</i>)	HM585552, HM585828, HM585968, HM585692

<i>Tetrastigma</i> <i>ceratopelatum</i> C.Y. Wu	Vietnam, Lao Cai (<i>J. Wen</i> 10870)	HM585558, HM585834, HM585974, HM585698
<i>T. cf. tuberculatum</i>	Indonesia, SE Sulawesi (<i>J. Wen</i> 10280)	HM585559, HM585835, HM585975, HM585699
<i>Tetrastigma cruciatum</i> Craib & Gagnep.	Thailand, Chiang Mai (<i>J. Wen</i> 7486)	HM585562, HM585838, HM585978, HM585702
<i>Tetrastigma curtisii</i> (Ridl.) Suesseng.	Indonesia, SE Sulawesi (<i>J. Wen</i> 10277)	HM585563, HM585839, HM585979, HM585703
<i>Tetrastigma delavayi</i> Gagnep.	Thailand, Chiang Mai (<i>J. Wen</i> 7443)	HM585565, HM585841, HM585981, HM585705
<i>Tetrastigma</i> <i>diepenhorstii</i> (Miq.) Latiff	Philippines, Luzon (<i>J. Wen</i> 8261)	HM585567, HM585843, HM585983, HM585707
<i>Tetrastigma eberhardtii</i> Gagnep.	Vietnam, Ninh Binh (<i>J. Wen</i> 10945)	HM585568, HM585844, HM585984, HM585708
<i>Tetrastigma ellipticum</i> Merr.	the Philippines, Luzon (<i>J. Wen</i> 8260)	HM585569, HM585845, HM585985, HM585709
<i>Tetrastigma erubescens</i> Planch.	China, Yunnan (<i>J. Wen</i> 10604)	HM585570, HM585846, HM585986, HM585710
<i>Tetrastigma funingense</i> C.L. Li	China, Yunnan (<i>J. Wen</i> 10579)	HM585574, HM585850, HM585990, HM585714
<i>Tetrastigma garrettii</i> Gagnep.	Thailand, Chiang Mai (<i>J. Wen</i> 7490)	HM585578, HM585854, HM585994, HM585718
<i>Tetrastigma glabratum</i> Planch.	Indonesia, West Java (<i>J. Wen</i> 10670)	HM585580, HM585856, HM585996, –
<i>Tetrastigma</i> <i>godefroyanum</i> Planch.	China, Hainan (<i>J. Wen</i> 6575)	HM585581, HM585857, HM585997, HM585719
<i>Tetrastigma</i> <i>gaudichaudianum</i> Planch.	Vietnam, Hoa Binh (<i>J. Wen</i> 10939)	HM585583, HM585859, HM585999, HM585721
<i>Tetrastigma</i> <i>hemsleyanum</i> Diels & Gilg	Vietnam, Ninh Binh (<i>J. Wen</i> 10792)	HM585585, HM585861, HM586001, HM585723
<i>Tetrastigma henryi</i> (Gagnep.) var. <i>henryi</i> Gagnep.	China, Yunnan (<i>J. Wen</i> 10518)	HM585586, HM585862, HM586002, HM585724
<i>Tetrastigma henryi</i> (Gagnep.) var. <i>mollifolium</i> W.T. Wang	China, Yunnan (<i>J. Wen</i> 10532)	HM585587, HM585863, HM586003, HM585725
<i>Tetrastigma</i> <i>heterophyllum</i> Gagnep.	Vietnam, Ninh Binh (<i>J. Wen</i> 10926)	HM585588, HM585864, HM586004, HM585726

<i>Tetrastigma hookeri</i> Planch.	Malaysia, Pahang (<i>J. Wen</i> 8381)	HM585589, HM585865, HM586005, HM585727
<i>Tetrastigma jinghongense</i> C.L. Li	China, Yunnan (<i>J. Wen</i> 8471)	HM585590, HM585866, HM586006, HM585728
<i>Tetrastigma laevigatum</i> Gagnep.	Indonesia, West Java (<i>J. Wen</i> 10131)	HM585591, HM585867, HM586007, HM585729
<i>Tetrastigma lanyuense</i> C.E. Chang	China, Taiwan (<i>J. Wen</i> 9404)	HM585593, HM585869, HM586009, HM585731
<i>Tetrastigma laoticum</i> Gagnep.	Vietnam, Guangnam (<i>J. Wen</i> 10969)	HM585595, HM585871, HM586011, HM585733
<i>Tetrastigma lawsoni</i> (King) Burkill	Singapore (<i>J. Wen</i> 7505)	HM585599, HM585874, HM586015, HM585737
<i>Tetrastigma laxum</i> Merr.	Philippines, Luzon (<i>J. Wen</i> 8314)	HM585602, HM585877, HM586018, HM585740
<i>Tetrastigma lenticellatum</i> C.Y. Wu ex W.T. Wang	China, Yunnan (<i>J. Wen</i> 10597)	HM585604, HM585879, HM586020, HM585742
<i>Tetrastigma loheri</i> Gagnep.	Indonesia, SE Sulawesi (<i>J. Wen</i> 10202)	HM585605, HM585880, HM586021, HM585743
<i>Tetrastigma napaulense</i> (DC.) C.L. Li	China, Xizang (Tibet 225)	HM585607, HM585882, HM586023, HM585745
<i>Tetrastigma napaulense</i> (DC.) C.L. Li	Nepal, Kathmandu (Z.-L. Nie & W.-D. Zhu 548)	HM585606, HM585881, HM586022, HM585744
<i>Tetrastigma obovatum</i> Gagnep.	China, Yunnan (<i>J. Wen</i> 10567)	HM585608, HM585883, HM586024, HM585746
<i>Tetrastigma obtectum</i> (Wall.) Planch.	China, Guizhou (Z.-L. Nie & Y. Meng 433)	HM585612, HM585886, HM586027, HM585750
<i>Tetrastigma pachyphyllum</i> (Hemsl.) Chun	Philippines, Luzon (<i>J. Wen</i> 8319)	HM585616, HM585891, HM586032, HM585753
<i>Tetrastigma papillosum</i> Planch.	Malaysia, Pahang (<i>J. Wen</i> 8401)	HM585617, HM585892, HM586033, HM585754
<i>Tetrastigma pedunculare</i> Planch.	Indonesia, SE Sulawesi (<i>J. Wen</i> 10281)	HM585620, HM585895, HM586036, HM585757
<i>Tetrastigma piscicarpum</i> (Miq.) Planch.	Indonesia, SE Sulawesi (<i>J. Wen</i> 10185)	HM585621, HM585896, –, HM585758
<i>Tetrastigma planicaule</i> Gagnep.	Vietnam, Ninh Binh (<i>J. Wen</i> 10904)	HM585622, HM585897, HM586037, HM585759
<i>Tetrastigma pyriforme</i> Gagnep.	Vietnam, Lam Dong (<i>J. Wen</i> 11006)	HM585623, –, HM586038, HM585760
<i>Tetrastigma retinervium</i> Planch.	Vietnam, Vinh Phuc (<i>J. Wen</i> 10920)	HM585625, HM585899, HM586040, HM585762

<i>Tetrastigma rumicispermum</i> Planch.	China, Yunnan (Tibet 2003)	HM585626, HM585900, HM586041, HM585763
<i>Tetrastigma serrulatum</i> Planch.	Vietnam, Lao Cai (J. Wen 10856)	HM585628, HM585902, HM586043, HM585765
<i>Tetrastigma serrulatum</i> Planch.	Thailand, Chiang Mai (J. Wen 7429)	HM585629, HM585903, HM586044, HM585766
<i>Tetrastigma siamense</i> Gagnep. & Craib	Chiang Mai (J. Wen 7485)	HM585630, HM585904, HM586045, HM585767
<i>Tetrastigma sichouense</i> C.L. Li	China, Yunnan (J. Wen 10547)	HM585631, HM585905, HM586046, HM585768
<i>Tetrastigma</i> sp. nov.	Indonesia, SE Sulawesi (G. Deden 976)	HM585640, HM585913, HM586055, HM585777
<i>Tetrastigma</i> sp.	China, Yunnan (J. Wen 8465)	HM585639, HM585912, HM586054, HM585776
<i>Tetrastigma</i> sp.	Indonesia, West Papua (J. Wen 10768)	HM585637, HM585910, HM586052, HM585774
<i>Tetrastigma strumarum</i> Gagnep.	Indonesia, Papua (J. Wen 10757)	HM585641, HM585914, HM586056, HM585778
<i>Tetrastigma tonkinense</i> Gagnep.	Thailand, Chiang Mai (J. Wen 7401)	HM585642, HM585915, HM586057, HM585779
<i>Tetrastigma trifoliolatum</i> Merr.	Malaysia, Selangor (J. Wen 8350)	HM585644, HM585917, HM586059, HM585781
<i>Tetrastigma triphyllum</i> (Gagnep.) W.T. Wang	China, Yunnan (J. Wen 10655)	HM585648, HM585921, HM586063, HM585783
<i>Tetrastigma tuberculatum</i> (Blume) Latiff	USA, Missouri Bot. Gard. (cult.) (J. Wen 6668)	HM585649, HM585922, HM586064, HM585784
<i>Tetrastigma tuberculatum</i> (Blume) Latiff	USA, Illinois (cult.) (J. Wen 7319)	HM585650, HM585923, HM586065, –
<i>Tetrastigma tuberculatum</i> (Blume) Latiff	Malaysia, Selangor (J. Wen 8335)	HM585651, HM585924, HM586066, HM585785
<i>Tetrastigma voinierianum</i> Pierre ex Pit.	USA, Illinois (cult.) (J. Wen 7320)	HM585652, HM585925, HM586067, HM585786
<i>Tetrastigma wangii</i> J. Wen	China, Yunnan (J. Wen 8455)	HM585653, HM585926, HM586068, HM585787
<i>Tetrastigma yunnanense</i> Gagnep.	China, Yunnan (cult.) (Z.-L. Nie 2003104)	HM585654, HM585927, HM586069, –
<i>Vitis aestivalis</i> Michx.	USA, South Carolina (J. Wen 10004)	HM585655, HM585928, HM586070, HM585788

<i>Vitis flexuosa</i> Thunb.	China, Yunnan (<i>J. Wen</i> <i>10647</i>)	HM585656, HM585929, HM586071, HM585789
<i>Vitis popenoei</i> J.L. Fennell	Mexico, Chiapas (<i>J.</i> <i>Wen 8724</i>)	HM585657, HM585930, HM586072, HM585790
<i>Vitis rotundifolia</i> Michx.	USA, Virginia (<i>J. Wen</i> <i>9972</i>)	HM585658, HM585931, HM586073, HM585791
