LEAF SHAPE EVOLUTION AND TAXONOMIC DIVERSIFICATION IN THE RADIATION OF PIPER (FAMILY PIPERACEAE) IN THE NEOTROPICS

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FACULTAD DE CIENCIAS
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Trabajo de Grado presentado como requisito
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RESUMEN

Título: EVOLUCIÓN DE LA FORMA DE LA HOJA Y DIVERSIFICACIÓN TAXONÓMICA EN LA RADIACIÓN DE PIPER (FAMILIA PIPERACEAE) EN EL NEOTROPICO[*]

Autor: Ángela Catherine Celis Tarazona[**]

Palabras claves: disparidad; evolución morfológica; morfometría de la hoja; tasa de diversificacion; radiación.

Descripción: La relación entre evolución morfológica y diversificación taxonómica es un campo activo de investigación que necesita de mejores herramientas y datos. Las *Piper* del Neotrópico son un linaje diverso de plantas que ofrece una gran oportunidad para evaluar esta conexión. Aquí, examinamos la relación entre diversificación taxonómica y morfológica usando variables continuas de la forma de la hoja para cuantificar la diversidad morfológica de las *Piper* Neotropicales. Examinamos patrones de evolución morfológica y diversificación taxonómica probando diferentes modelos de evolución de caracteres y diversificación taxonómica en la filogenia más completa a nivel de especie para *Piper*. Cuantificamos la disparidad morfológica a partir de variables de la forma de la hoja medidas de 120 especies de *Piper*. Las estimaciones de las edades de divergencia y la tasa de diversificación revelaron que la diversidad actual del género resulta de una tasa constante de diversificación y una radiación reciente de los subclados *Radula* y *Macrostachys*. **La diversidad morfológica dentro de los subclados de *Piper* es alta y ha permanecido constante en el tiempo**. La diversificación taxonómica y morfológica de las *Piper* Neotropicales están desacopladas, lo cual muestra que la diversificación taxonómica no ha estado limitada por la disponibilidad de espacio ecológico o fenotípico.

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[*] Tesis de grado

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ABSTRACT

Title: LEAF SHAPE EVOLUTION AND TAXONOMIC DIVERSIFICATION IN THE RADIATION OF PIPER (FAMILY PIPERACEAE) IN THE NEOTROPICS[*]

Author: Ángela Catherine Celis Tarazona[**]

Keywords: disparity; diversification rate; leaf morphometrics; morphological evolution; radiation.

Description: The association between morphological evolution and taxonomic diversification is a field of active research that needs of better tools and data sets. The Neotropical *Piper* is a diverse lineage of plants which offers a great opportunity to evaluate this connection. Here we examined the relationship between taxonomic and morphological diversification using continuous variables of leaf shape to quantify the morphological diversity of the Neotropical *Piper*. Patterns of morphological evolution and taxonomic diversification were examined testing different models of trait evolution and taxonomic diversification in the most complete species-level phylogenetic hypothesis produced to date for *Piper*. Morphological disparity was quantified from continuous leaf variables of 120 species of the Neotropical clade of *Piper*. Estimates of age divergence and taxonomic diversification revealed that present-day *Piper* taxonomic diversity results from a constant accumulation of lineage and the recent radiation of *Radula* and *Macrostachys* subclades. Morphological diversity within *Piper* subclades has diversified highly and remained constant over time. Taxonomic diversification of the Neotropical *Piper* is decoupled from morphological evolution, showing that taxonomic diversification has not been limited by ecological nor phenotypic space available.

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[*] Bachelor Thesis

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INTRODUCTION

The Neotropical region comprises the greatest number of flowering plant species worldwide (Gentry, 1982; Thomas, 1999). How this plant diversity has been generated and maintained is a complex question with biotic and abiotic factors involved (Antonelli and Sanmartín, 2011). One way to approach this question is to examine pantropical lineages that have high Neotropical diversity (Hughes et al., 2013). The northern Andes and southern Central America bear unusually species-rich genera, as *Anthurium*, *Piper* or *Pleurothallis* which are predominantly epiphytic, shrubby or herbaceous (Gentry, 1982). The high diversity of these genera has been attributed to an "explosive" speciation during the Andean orogenesis at the Neogene (Gentry, 1989; Quijano-Abril et al., 2006). Contrary to taxonomic diversity, morphological diversity of Neotropical species-rich genera has been poorly quantified (Luebert et al., 2011). The relationship between the evolution of morphological diversity and taxonomic diversification is a useful comparative approach to identify general patterns among evolutionary radiations (Harmon, et al., 2003). Most studies about the evolution of morphological diversity and its relationship with diversification have been carried out in animals revealing new insights in taxonomic diversification (e.g. Derryberry et al., 2011; Blankers et al., 2013; Rabosky et al., 2013; Hipsley et al., 2014). However, very little is known about this relationship in plants and much less in Neotropical plants; mainly because the genera involved are commonly large and taxonomically difficult with many undescribed species (Gentry, 1989).

Plants of the genus *Piper* L. (Piperaceae) offer a unique opportunity to study the patterns that underlie diversification processes in the Neotropical region because they add to nearly 2000 species, have a wide geographical representation in the Neotropics (1300 sp.) as in the Paleotropics (700 sp.), and there is a comprehensive reconstruction of phylogenetic relationships (Quijano et al., 2006; Jaramillo et al., 2008). Despite being a lineage with a long-standing history in Neotropics (Martínez et al. 2015), *Piper* provides a noteworthy example of an increase in diversification rate at the base of the angiosperms (Jaramillo et al., 2004). Additionally, it has been suggested that *Piper* species show relatively little morphological diversity in comparison with the high species richness in the genus (Burger, 1972; Marquis, 2004). The premise is that the large diversification in Neotropical *Piper* may have occurred relatively recently, thus little time has been available for morphological diversification (Gentry 1989). All these assumptions have not been tested and make *Piper* a compelling system to study for the evolution of morphological diversity in Neotropical plants.

We chose leaf shape to analyze morphological diversification of Neotropical *Piper.* Leaf shape is a highly labile trait which is responsive to a range of biotic and abiotic factors that enabled us to study the fine tuning of the leaf to its ecological conditions over short and evolutionary time spans (Nicotra et al., 2011). Although diversity of vegetative characters represents a significant portion of morphological diversity in plants, little is known about its diversification in Neotropical plant lineages in comparison with diversification of reproductive characters (Luebert et al., 2011). Moreover, leaf shape is often measured in discrete categories that may omit a significant portion of the morphological variation. For the morphological analysis presented, we implemented quantitative variables that describe leaf shape and allowed us to estimate morphological disparity, which is the distribution of taxa into vast or limited regions of the morphospace (Erwin, 2007).

Here, we studied the association between evolution of morphological diversity and taxonomic diversification in Neotropical *Piper* using a comparative phylogenetic approach. This analysis enabled us to test three potential relationships between morphological disparity and taxonomic diversity: a constrained disparity with morphological diversification decoupled from taxonomic diversification, a rapid increase in disparity exceeding initial taxonomic diversification, and concordant increases in morphological disparity and taxonomic diversity (Foote, 1993; 1996; Wesley-Hunt 2005). To realize the Analysis we first estimated a time calibrated phylogeny improving the sampling of ITS sequence for Neotropical *Piper* (Appendix 1.). To provide insight into the underlying causes of taxonomic diversification, we used likelihood methods for detecting temporal shifts in diversification rates and lineage through time plots (Nee et al. 1992). Then we identified general patterns of phenotypic evolution using likelihood models of continuous trait evolution (Pagel 1999) and disparity through time plots (Harmon et al. 2003).

1. MATERIALS AND METHODS

1.1 DIVERGENCE TIME ESTIMATION

We estimated a time-calibrated phylogeny using BEAST v2.1.2, a software framework for Bayesian evolutionary analysis that includes the estimation of gene phylogenies and associated divergence times in the presence of calibration information from fossil evidence (Bouckaert et al., 2014). The molecular sequences and model parameters were set in BEAUti, a utility program that allows the creation of BEAST XML input files. We used a sampling of unique sequences of the internal transcribed spacer (ITS) for 302 species from Neotropics (Appendix 1; Jaramillo, in prep.). We performed two independent runs of 100 million generations setting a HKY+G substitution model, which was selected using jModeltest 2.1.5 (Darriba et al., 2012), an uncorrelated lognormal relaxed clock model, and a Yule model as tree prior (Martínez et al., 2015; Molina-Henao et al. in prep). The phylogeny was calibrated using the register of *Piper alatabaccum* Trel. & Yunck. Neogene seeds and the fossil leaves of *P. margaritae* Martínez-A., which are related to the *Piper* subclade *Ottonia* Spreng (Callejas, 2002) and *Schilleria* Kunth (Martínez et al., 2015), respectively. As Neogene seeds match with a high correspondence to *P. alatabaccum,* this calibration point was located at the stem of *P. alatabaccum*, with a log-normal distribution, a lower age of 6.5 Ma, and a standard deviation equal to one. As morphological analyses located the fossil leaves of *P. margaritae* within the clade Schilleria, we associated it with the stem of this clade. It was set with a log-normal distribution, a lower age of 67 Ma, and a standard deviation equal to one. Since Piperaceae has a poor fossil record, we also incorporated a secondary calibration point as the maximal age of origin of the genus at 135 Ma, with a normal distribution and a standard deviation equal to one. This age was set according to the age of origin of Piperales (Wikström et al., 2003; Moore et al., 2007; Bell et al., 2010). Output log files were combined in LogCombiner (Drummond and Rambaut, 2007) and evaluated in Tracer 1.6 (Drummond and Rambaut, 2007) to confirm that the replicate runs converged, that all parameters met effective sample size values (n>200) and to identify and discard the burn-in. BEAST also produces a posterior sample of phylogenetic time-trees, along with its sample of parameter estimates. We summarized these in a maximum clade credibility tree (MCC) using TreeAnnotator (Drummond & Rambaut, 2007).

1.2 TAXONOMIC DIVERSIFICATION ANALYSES

All diversification analyses were performed in R (R Development Core Team 2014) implementing functions from the libraries APE (Paradis et al., 2004), GEIGER (Harmon et al., 2008), LASER (Rabosky, 2006a), and Phytools (Revell, 2012). To test whether diversification rates have changed over time we fitted onto the time-calibrated phylogeny, from the BEAST analysis, the following maximum-likelihood models based on the birth-death process: pure-birth (yule), birth–death (BD), Yule with two rates (yule2rates), Yule with three rates (yule3rates), Yule with four rates (yule4rates), Yule with five rates (yule5rates), logistic diversity-dependent (DDL), exponential diversity-dependent (DDX), a model with exponentially declining speciation rate through time (constant extinction; SPVAR), a model with exponentially increasing extinction rate through time (constant speciation; EXVAR), and a model with varying speciation and extinction rates through time (BOTHVAR). These models are further explained elsewhere (Rabosky et al., 2007; Rabosky and Lovette, 2008). To test whether the best rate-constant model or the best rate-variable model better fits the data we computed the test statistic:

$$\Delta \mathrm{AICc} = \mathrm{AIC}_{\mathrm{Constant}} - \mathrm{AIC}_{\mathrm{Variable}},$$

where $\mathrm{AIC}_{\mathrm{Constant}}$ is the AIC (Akaike Information Criterion) score of the best rate-constant model and $\mathrm{AIC}_{\mathrm{Variable}}$ is the AIC score of the best rate-variable model. We considered an increase in model fit to be significant when the reduction in AIC score in the rate-variable model was ≥ 4 (Burnham and Anderson, 2002; Rabosky, 2006b).

Temporal variation in diversification rates was visualized with semi-logarithmic lineage-through-time plots (LTT; Nee et al., 1992). In the LTT plot the x-axis represents time since the first bifurcation, the y-axis the number of lineages that have originated in that range of time, and the slope of the curve reflects the per-lineage rate of diversification. If the rate is constant through time, the LTT plot would appear to be a straight line with stochastic movement (Nee et al., 1992). The observed LTT was calculated using the MCC tree and its 95% confidence interval was calculated from the LTT plots using the last 1000 trees of the posterior distribution of BEAST analyses.

To detect lineage-specific shifts in diversification rates we used MEDUSA, a comparative method based upon the AIC, for detecting multiple shifts in birth and death rates on an incompletely resolved phylogeny (Alfaro et al., 2009). MEDUSA integrates phylogenetic information about the timing of splits along the backbone of a tree with taxonomic richness data to estimate rates of speciation and extinction (Alfaro et al., 2009). As we have an incomplete phylogeny but information about richness inside each subclade, we pruned our 322-taxon timetree down to eight tips representing major taxonomic divisions inside Neotropical clade: *Radula* Miquel 450 species, *Peltobryon* Klotzsch 100, *Macrostachys* Miquel 250, *Pothomorphe* C. DC. 10, *Schilleria* Kunth 200, *Enckea* Kunth 120, *Ottonia* Spreng 50, and *Piper cinereum* C. DC. and *Piper sanctum* (Miq.) Schltdl. ex C. DC. (Jaramillo et al., 2008). AIC values corrected for small sample sizes (AICc) were used for model comparisons. The threshold for retaining additional rate shifts was an improvement in AICc score of 4 units or greater (Burnham and Anderson, 2002).

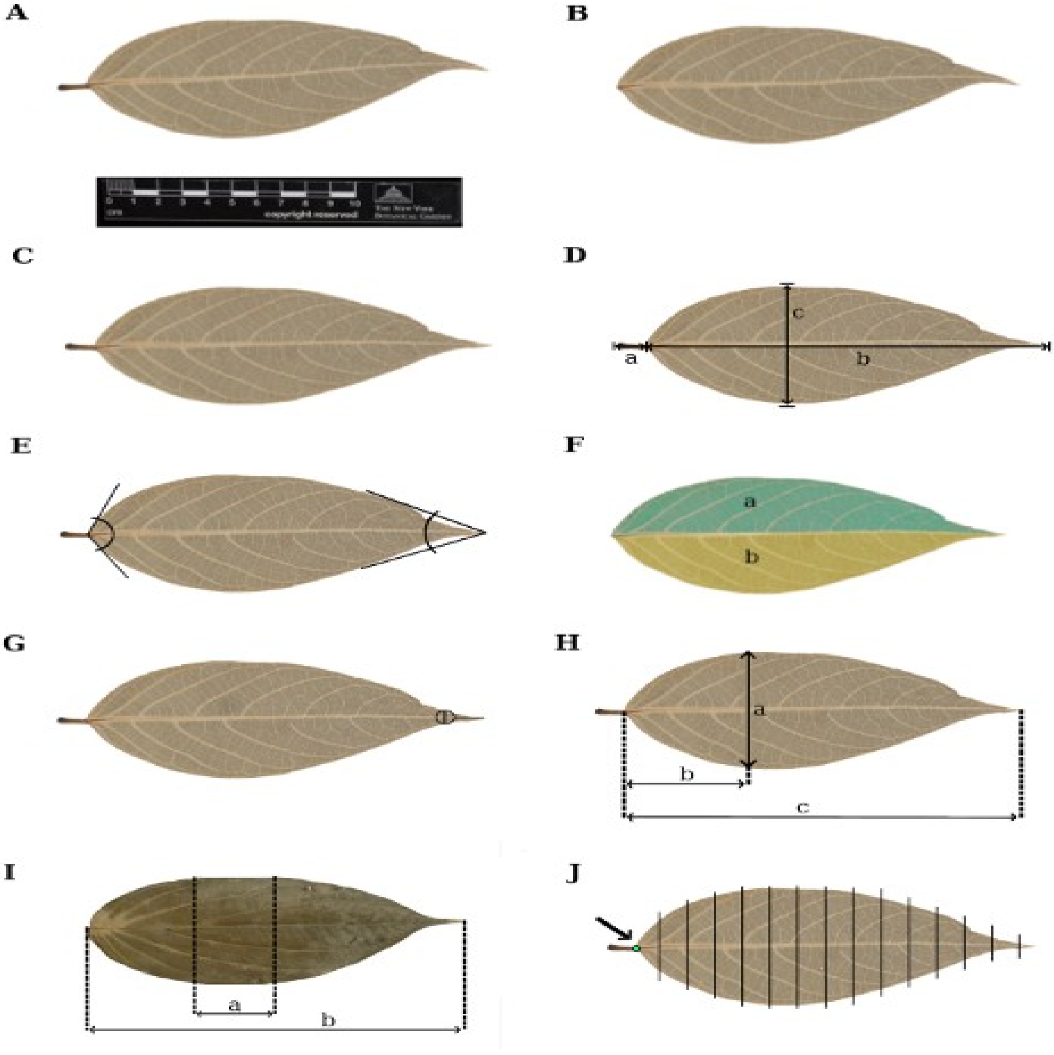
1.3 MORPHOLOGICAL DISPARITY AND MORPHOLOGICAL EVOLUTION ANALYSES

Morphological variation was measured using 10 variables that describe leaf blade shape and size (Fig. 1): (1) petiole length, the length of the stalk attaching lamina to the stem; (2) lamina elongation, the ratio of lamina length to lamina width (L/W; Niinemets et al., 2007); (3) area of lamina; (4) apex angle and (5) base angle, which were measured based on Ellis et al., (2009); (6) drip-tip length, which was defined as the distance from the point of intersection between a circle and the leaf midrib to the distal end of the drip tip, the circle was drawn with a diameter equal to the length of the line connecting the inflection points of the apex margin (Meng et al., 2014); (7) moment of inertia, which indicates leaf mass distribution along the lamina (M. Torres and A. Celis, in prep.); (8) asymmetry area, the ratio of top area to bottom area of lamina divided across the midvein; (9) oblongation index, which indicates the proportion of the widest area relative to leaf length equation; and (10) obovacity index, which estimates the position of the widest point of lamina equation. We took the measurements from photos of museum specimens using a custom program (M. Torres, in prep.) made in Matlab (MathWorks, 2012). This program digitally unbends each leaf before calculating measurements to avoid measurement error related with leaf bending. We collected data for 120 species in the phylogeny, which represent at least 23% of the species in each of the six main Neotropical subclades. No morphological data pertaining to 182 species were available and they were thus excluded from the analyses. On average, we measured three leaves per specimen and three specimens per species, using the average per species in further analyses.

A Principal Components Analysis (PCA) was calculated on the correlation matrix of the shape parameters. The first three shape PCs were used to investigate the history of morphological evolution. Three models of continuous trait evolution were fitted to PCs and the time-calibrated phylogeny from the BEAST analysis (Pagel, 1999): random walk model (BM model), a model of constrained trait evolution toward an optimum (Ornstein-Uhlenbeck model) and a model of deceleration ($\delta<1$) or acceleration ($\delta>1$) of trait evolution through time (Delta model). AICc scores were estimated to compare the fit of these models. The threshold to accept a model that better fits trait evolution than a BM model was an improvement of AICc score in 4 units or greater (Burnham and Anderson, 2002).

We measured the time course of morphological diversification using disparity through time plots (DTT) implemented in Geiger. Disparity was calculated from average pairwise Euclidean distances between species, a variance-related method of estimating the dispersion of points in multivariate space that is insensitive to sample size (Ciampaglio et al., 2001). We also calculated morphological disparity index (MDI), which is the area between the observed and expected median disparities. Negative values of MDI indicate that disparity through time is less than predicted under BM and that most variation is partitioned as among basal clades. Such a pattern indicates that clades tend to occupy different regions of morphological space, which is a common feature of adaptively radiating lineages (Harmon et al., 2003). On the other hand, positive values indicate that subclades converge on a pattern whereby overall morphological disparity is partitioned among members within each subclade. As overdispersion due to incomplete sampling can influence the calculation of disparity, we restricted our analysis to the first 80% of the time-calibrated phylogeny (Muschick et al., 2012; Near et al., 2012).

Figure 1. Description of leaf variables measured. (A) Leaf and scale from collection voucher of Piper alatabaccum. (B) Leaf area. (C) Leaf straightened. (D) Petiole length equals a; length-width ratio equals b over c, where b = leaf length and c = leaf width. (E) Base and tip angles. (F) Asymmetry area: is the ratio between a and b. (G) Drip-tip length: is the distance between the most distal point of leaf tip and the perimeter of a circle drawn across the inflection points of the tip margin. (H) Obovacity index equals to b over c multiplied by 100, where a = maximum leaf depth, b = length at maximum leaf depth, c = leaf length. (I) Oblongation index equals to a over b multiplied by 100, where a = length with same depth, b = leaf length. (J) The moments of inertia of the sample are approximated as the moments of inertia of rectangles with height equal to a and width equal to b; the arrow points the rotational axis of the lamina.



2. RESULTS

2.1 DIVERGENCE TIME ESTIMATION

The maximum-clade credibility tree retrieved a monophyletic clade that represents the Neotropical species of *Piper* (Fig. 2). The Neotropical clade split from Asian and South Pacific clades 135 Ma in the Early Cretaceous and started to diversify 116 Ma. Diversification of the Neotropical clade led to seven subclades that form two main monophyletic groups. The first group comprises the *Schilleria*, *Ottonia*, and *Enckea* subclades that diverged 106 Ma and have in common that the flowers are loosely arranged on the inflorescences. The second group comprises the *Pothomorphe*, *Peltobryon*, *Macrostachys*, and *Radula* subclades, which diverged 95 Ma and have inflorescences with tightly congested flowers forming banding patterns. Roughly speaking, subclades within *Piper* originated early, but extant species inside each subclade originated more recently in the last 30 Ma.

2.2 TAXONOMIC DIVERSIFICATION ANALYSES

The best-fitting model was the yule4rates model with a reduction in AIC score from the best constant-rate model (Yule model) of 24.8 (Table 1). The yule4rates model detected three shift points in the overall diversification rate, two of which indicated an abrupt increase in the diversification rate. The first shift occurred 9.3 Ma, when the speciation rate increased from 0.05 to 6.8 lineages/Ma, next to this age occurred the second shift 9.3 Ma, the speciation rate decreased from 6.8 to 0.04 lineages/Ma. At the third shift 1.8 Ma, the speciation rate decreased from 0.04 to 0.009 lineages/Ma. This rapid reverse indicates that the yule-n-rate models tend to over-fit the model with the majority number of parameters. We performed again the yule-n-rate models fixing the number of shifts to consider (ints). The result was that yule2rates model was the best-fitting model, the shift point occurred 1.2 Ma with a slowdown in diversification rate from 0.05 to 0.005. Using MEDUSA package we detected two lineage-specific shifts from the background diversification rate.

These shifts led to two exceptionally species-rich Neotropical subclades, Radula and *Macrostachys*. The first shift occurred within the base of the *Radula* subclade 51 Ma, when the net diversification rate was 0.06 (ΔAICc=342.23). The second shift occurred within the base of *Macrostachys* subclade at 29 Ma, when the net diversification rate was 0.08 (ΔAICc=375.25). The LTT plot showed a nearly constant pattern of lineage accumulation through time (Fig. 3), nonetheless a slightly increment in net diversification rate was observed around 30 Ma which is consistent with the shift points found by MEDUSA.

2.3 MORPHOLOGICAL DISPARITY AND MORPHOLOGICAL EVOLUTION ANALYSIS

The first three PCs explained 74% of the variation in shape, size, and area distribution along the longitudinal axis for *Piper* leaves. The first PC explained 43% of variation and was chiefly dominated by shape and showed a range of leaves that go from elongated, elliptic, with acute tip and base angles, and with short petioles, to short, cordate, with obtuse tip and base angles, and with long petioles. The second PC explained 18% of variation and was chiefly dominated by leaf size, and the third PC explained 11% of variation and was chiefly dominated by obovacity index and asymmetry area. The time course of morphological diversification showed that relative disparity-through-time was greater than expected under the null model of Brownian motion throughout the radiation of Neotropical *Piper* (Fig. 4). This result was quantitatively supported by a significantly positive MDI value (0.4). It means that morphological diversity was partitioned within, instead of among subclades; creating an overlap in morphospace occupation (Fig. 5). This pattern is likely to be the result of a time-homogeneous Brownian motion process of morphological evolution in agreement with the model analysis to the first three PCs, that were best-fit by the BM model (Table 2).

Table 1: Akaike Information Criterion values from diversification models fitted to the branching times derived of the maximum credibility clade phylogeny, sorted from the best- to the least-fit model.

Model	Log likelihood	AICc[a]	ΔAIC[b]
Yule4rates[c]	203.70	-393.40	0
Yule5rates	205.68	-393.37	0.03
Yule3rates	199.86	-389.73	3.67
Yule2rates	197.06	-388.12	5.28
DDL[d]	188.92	-373.83	19.57
Yule[e]	185.29	-368.58	24.82
DDX[f]	186.06	-368.11	25.29
BD[g]	185.29	-366.58	26.82
SPVAR[h]	185.69	-365.39	28.01
EXVAR[i]	185.00	-364.01	29.39
BOTHVAR[j]	185.69	-363.39	30.01

Notes: [a] Second order information criterion. [b]Difference in AIC values between each model and the overall best-fit model. [c] Yule-n-rate models are multi-rate variants of the Yule model. [d,f] Density-dependent speciation rate models, "DDL" and "DDX" fit logistic and exponential variants of the density-dependent speciation rate model. [E] Pure birth model, it only estimates speciation (birth) rate. [g] Birth-death model, it estimates the net diversification rate (speciation rate minus the extinction rate). [hij] Time varying speciation and extinction models, SPVAR is a model with an exponentially declining speciation rate through time and constant extinction, EXVAR is a model with an exponentially increasing extinction and constant speciation, and BOTHVAR is a model where both speciation and extinction rates can vary through time.

Figure 2. Maximum clade credibility phylogeny. Bayesian estimate of phylogenetic relationships and divergence times among species of Neotropical Piper (family Piperaceae) as inferred from ITS sequence. Bars at nodes indicate the 95% highest posterior density for the inferred divergence time estimates. The numbers below subclades names correspond to the number of species within.

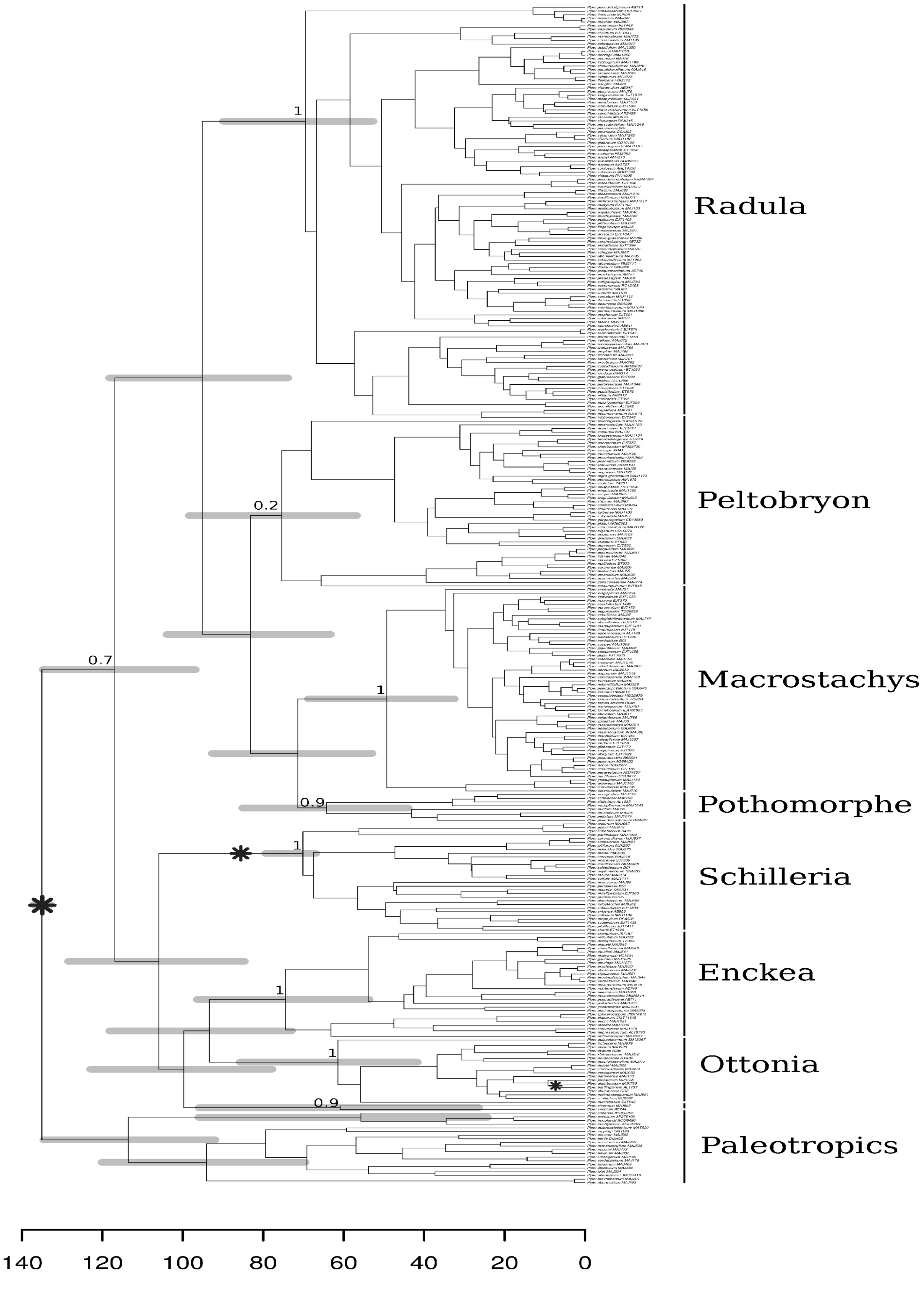


Figure 3. Lineage accumulation over time in the Neotropical Piper radiation. The continuous line represents the number of lineages through time for the maximum clade credibility tree. The gray shaded area is the 95% confidence interval calculated from the last 1000 trees of the posterior distribution of BEAST analysis. The dashed line indicates the expected number of lineages under a constant-rate model of diversification with no extinction.

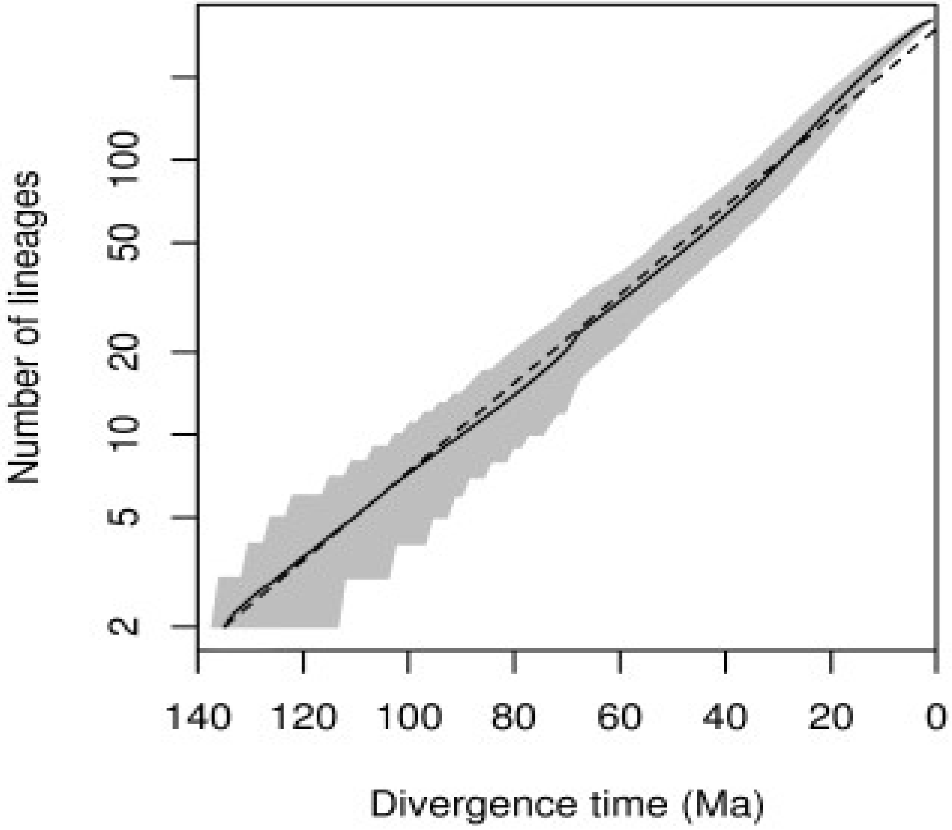


Table 2: Summary of ΔAICca for three models of trait evolution for each morphological principal component.

Model	PC1	PC2	PC3
Bm[b]	892.4	746.9	734.2
	0[a]	0	0
OU[c]	894.5	747.7	736.1
	11.2	0.8	1.9
Delta[d]	905.7	754.7	735.9
	13.3	7.8	1.7

Notes: [a]Difference in second order information criterion values between each model and the overall best-fit model. [b]Brownian Motion model. [c]Ornstein–Uhlenbeck model. [d]Delta model.

Figure 4. Relative disparity through time. Disparity values closer to 1 indicate that most variation is found within subclades and values closer to 0 indicate that variation is partitioned among subclades relative to the entire clade. The relative time scale go from 116 Myr (0.0) to 23 Myr (0.8). Solid line indicates actual disparity, dashed line indicates median expected disparity and gray area indicates expected disparity for a sample of 100 simulations based on a Brownian motion model.

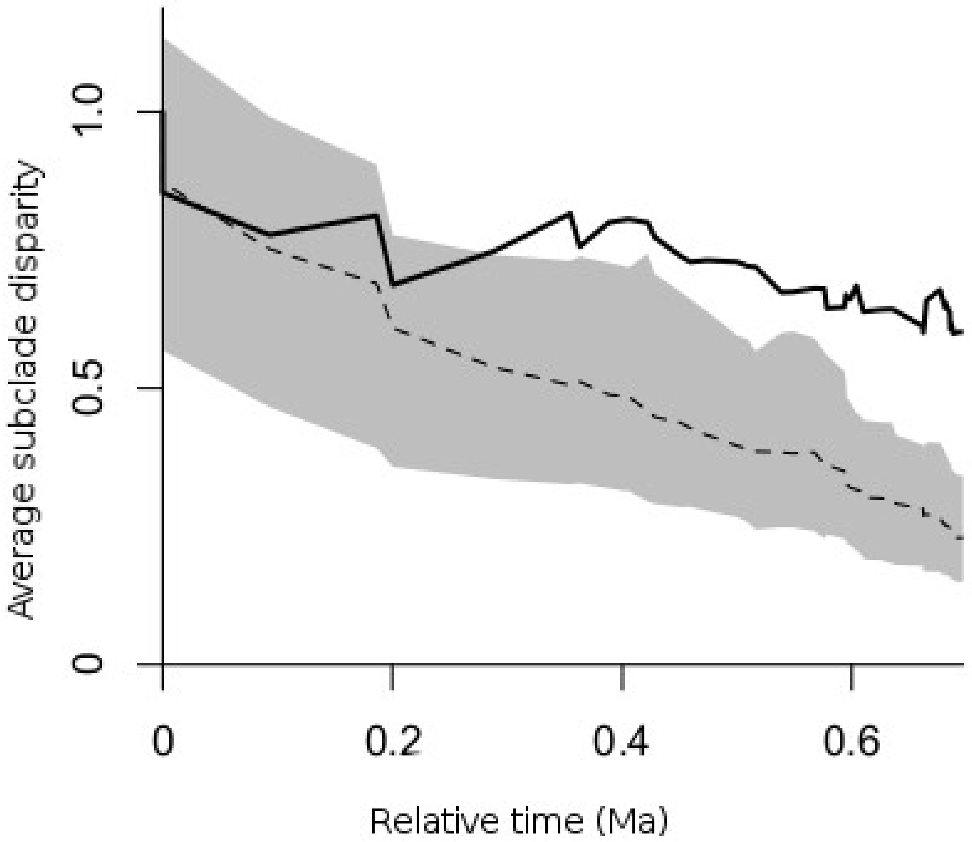
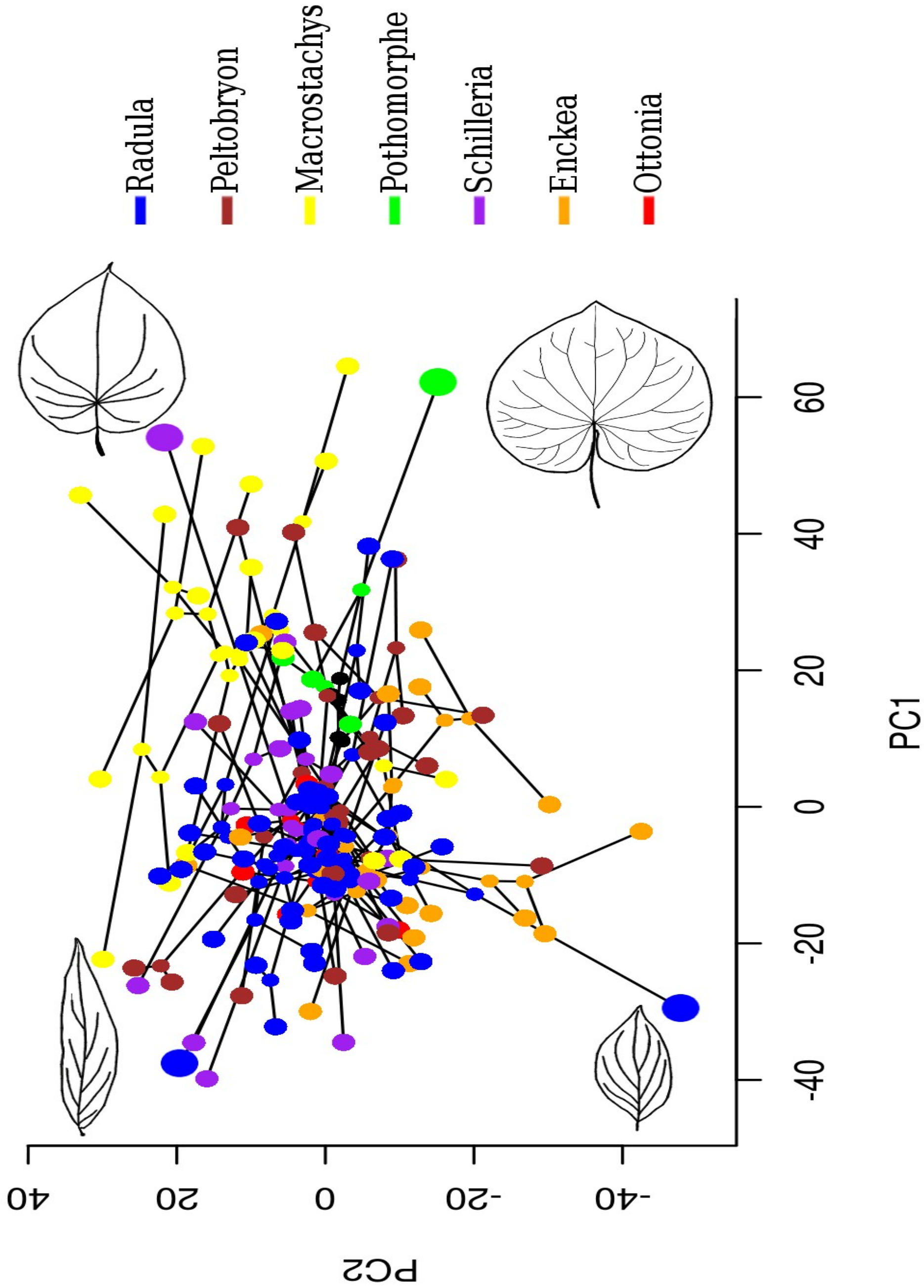


Figure 5. Piper leaf shape morphospace. Phylomorphospace plot based on morphological variables that quantify size, area, shape, tip and base angles, elongation, and asymmetry of leaf lamina. Black lines connect related taxa through hypothetical ancestors. Species colors match Neotropical subclades. The biggest points are the species represented.



3. DISCUSSION

We presented an analysis of the general patterns in the relationship between leaf morphological evolution and taxonomic diversification in the Neotropical clade of *Piper*; an approach that has not been tested in Neotropical plants. Our results show that extant taxonomic diversity results from a constant lineage accumulation and a recent radiation in *Radula* and *Macrostachys* subclades. In contrast to what has been suggested—that *Piper* morphological diversity is low compared to its taxonomic diversity (Marquis et al., 2004) —we found that morphological diversification was high during taxonomic diversification in comparison to morphological disparity of Neotropical groups as Furnariidae and Arini (Derryberry et al., 2011; Schweizer et al., 2014). The pattern of diversification of Neotropical *Piper* is consistent with a morphological diversification that outstrips taxonomic diversification with large morphological steps within subclades, rather than among subclades.

The Neotropical *Piper* not only diverged early in the evolutionary history of the genus but also radiate recently around the last 30 Ma. Gentry (1989) suggested that species-rich genera like *Piper* resulted from an "explosive" speciation within the last two million years, based on the high number of species and local endemisms observed in the genus. Recent analysis of the patterns of diversification of *Piper* support the scenario of a recent radiation in the Neotropics which better resembles a cradle model than a museum model of taxonomic diversification (Smith et al., 2008; Martínez et al. 2015). These two standing hypotheses, cradle and museum models, explain biodiversity in the Neotropics: the museum model depicts the gradual accumulation and/or preservation of lineages over time (Mittelbach et al., 2007) and the cradle model describes the recent and rapid accumulation of species. These models are often presented as temporal alternatives, however, their predictions are not mutually exclusive (McKenna and Farrell 2006). Indeed, our data suggest that diversification of Neotropical *Piper* exhibits characteristics of cradle and museum models. Martínez et al. (2015) found that the pattern of taxonomic diversification of *Piper* genus better resembles a cradle model. The cradle model that they used represents a scenario in which the increase in speciation rates correlates with the Andean uplift. We used a maximal age of origin of *Piper* older than that used by Martínez et al., and we got an overall difference of 21 Ma in the divergence ages for major subclades. Our divergence analysis converged on estimates for the origin of the angiosperms (180–140 Ma) which have indicated that Piperales is an early lineage (Bell et al., 2010). In contrast to Martínez et al., we found that the overall taxonomic diversification better resembles to a museum model of constant species accumulation. However, we used MEDUSA and specifically detected that diversification rate increased at the base of the *Macrostachys* and *Radula* subclades, with a 127% and a 55% increases from the background rate, respectively. The number of species that arose in *Macrostachys* (~250 sp.) and *Radula* (~450 sp.) at the last 30 Ma, makes the radiation of Neotropical *Piper* comparable to the explosive radiation in *Brathys* clade of *Hypericum* (149 species; 13.9 Ma; Nurk et al. 2013), and even greater than some lineages from Páramo ecosystems (*Arcytophyllum*, *Lysipomia*, *Valeriana*; Madriñan et al., 2013). We propose that Neotropical forests have been at the same time evolutionary cradles and museums of *Piper* diversity.

Diversification is decoupled from morphological disparity according to the general hypothesis regarding evolutionary radiations that potentially links these patterns. This hypothesis suggests that clades that showed early bursts of species diversification would also show greater among-subclade than within-subclade disparity in their morphology, mainly because groups with early bursts of radiation have filled or occupied the available niche space, which slows subsequent radiation (Harmon et al. 2003). In the contrary case, the clades that exhibit a constant diversification rate distribute most of their morphological diversity within subclades rather than among subclades. Neotropical *Piper* do not show an early burst of diversification; they partition more of their morphological variation within-than among-subclades, and their leaf shape traits appear to be evolving according to a random walk process that have continued through time. Thus our results support the general predictions of Harmon et al. (2003) and suggest that the radiation of Neotropical *Piper* continue filling the available morphospace and ecological space within subclades.

Neotropical subclades exhibit an extensive morphological diversification within them rather than among them. Harmon et al. (2003) have suggested that clades which partition morphological diversity within- rather than among-clades tend to substantially overlap each other in morphospace occupation. The taxonomists of the *Piper* genus suggest that Neotropical subclades differ well morphologically, but that within each subclade the morphological differences are not as clear (Jaramillo and Callejas, 2004). We found that Neotropical subclades substantially overlap each other in the morphospace of the *Piper* leaf shape, specially in leaves of median size, laminas ovate to elliptic, acuminate apices, and short petioles. However, subclades as *Macrostachys* and *Enckea* tend to occupy specific regions whereas *Radula* expand in most of the possible space. A greater variety of leaf shapes allowed *Piper* to specialize to the newly formed habitats during Andean uplift. For example, we observed that leaf shapes were arranged in a continuum: one end represents small leaves with short petioles and acute tips, the other end represents big leaves with long petioles and drip-tips. Traits like higher specific leaf area and longer leaf drip tips are associated with habitats with high precipitation (Meng et al. 2014). As discussed by Martínez et al. (2015) there is a temporal relation between the diversification of *Piper* and the Andean uplift. The rise of the Andes not only affected the diversity creating newly formed montane habitats (e.g. Madriñan et al. 2013), it also altered regional climate, nutrient deposition, and riverine systems of lowland habitats (Hoorn et al. 2010; Antonelli and Sanmartín, 2011). *Piper* reaches its highest diversity in the lowlands of the Neotropical region (Gentry 1990) where high precipitation is a selective factor (Meng et al. 2014). We hypothesize that *Piper* species adapted to lowland rainfall forest have increased variation and evolved methods like higher specific leaf area and longer leaf drip tips since the beginning of the Miocene in groups like *Macrostachys*. To understand what happened in groups as *Enckea* and *Radula*, we need better information about distribution and ecomorphological traits within each subclade.

The time-calibrated phylogeny used here for the diversification analysis was constructed employing a sample of 302 sequences of Neotropical species of *Piper*. This sampling constituted the most complete selection of Neotropical *Piper*, it containing 23% of Neotropical diversity and at least a 15% of diversity within each subclade. However, the sampling is small considering the number of extant Neotropical species of *Piper* (~1300). Thus the *Piper* calibrated phylogeny could yield a false signature of a increase in diversification called "pull of the present" (Nee et al. 1994). If true, then including more lineages may erase the recent rate shift. Even so, methods as implemented in LASER and MEDUSA packages show that speciation and extinction rates can be estimated even if the phylogeny is incomplete as long as we do the correct assumptions about how the sampling process was performed (Stadler and Bokma, 2013).

To better understand the extraordinary diversity of *Piper* (~2000 species; Quijano et al., 2006) we need further research into the evolutionary processes at the infrageneric level. In this study, subclades within Neotropical *Piper* exhibited substantially different patterns of lineage evolution. We identified two radiations: one at the base of *Radula* ~50 Ma, the other at the base of *Macrostachys* 29 Ma. To characterize each of these radiations, further sampling of ecomorphological traits within each subclade is necessary. The analytical framework of the study of taxonomic and morphological diversification presented here may help to understand the evolutionary history of the handful of species-rich lineages that have been evolving in the Neotropical region.

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APPENDICES

APPENDIX A. List of collection vouchers and GenBank accessions for species used in phylogenetic analysis. ***Taxon***; Voucher specimen; Collection locale; Herbarium; GenBank accession ITS sequence; x = sequence disponible with M. A. Jaramillo. A list of collector's abbreviations is provided at the end of the appendix.

***Piper abalienatum* Trel.**; MAJ 552; Mexico; US; EU581075.1. ***P. abbreviatum* Opiz**; MAJ 203;Philippines;DUKE;EU581454.1. ***P. acutifolium* Ruiz & Pav.**; MAJ 1250; Bolivia; MO; x. ***P. adenandrum* (Miq.) C. DC.**; EJT 557;French Guiana;MU;EU581077.1. ***P. aduncum* L.**; MAJ 76; Colombia; DUKE; AF275157.1. ***P. aequale* Vahl**; DSA 331; Costa Rica; MO; x. ***P. aereum* Trel.**; IAC 2213; Costa Rica; MU; EF056220.1. ***P. alatabaccum* Trel. & Yunck.**; MJK 730; Brazil; MG; EU581343.1. ***P. albispicum* C. DC.**; MAJ 388; Vietnam; DUKE; AY572317.1. ***P. albozonatum* C. DC.**; MAJ 1319; Colombia; CUCV; x. ***P. amalago* L.**; MAJ 1273; Dominican Republic; HUA; x. ***P. amplum* Kunth**; MAJ 785; Brazil; RB; EU581095.1. ***P. anceifolium* Kunth**; EJT 184; Colombia; MU; EU581272.1. ***P. anisum* (Sprengel) Angely**; MAJ 920; Brazil; RB; EU581097.1. ***P. anonifolium* Kunth**; AL 1242; Brazil; MG; EU581084.1. ***P. arboreum* Aubl**; MAJ 1163; Bolivia; HUA; x. ***P. arieianum* C. DC.**; MAJ 638; Colombia; MU; EU581108.1. ***P. artanthe* C. DC.**; MAJ 83; Colombia; DUKE; EU581109.1. ***P. asymmetricum* C. DC.**; DSA 333; Costa Rica; MO; x. ***P. attenuatum* Buch.-Ham. ex Miq.**; LJM 061416; China; PE; EU581110.1. ***P. augustum* Rudge**; MAJ 122; x; DUKE; DQ882203.1. ***P. aulacospermum* Callejas**; SM 18087; French Guiana; HUA; EU581112.1. ***P. auritifolium* Trel.**;CD 10877; Costa Rica; SRP; EU581113.1. ***P. auritum* Kunth**; MAJ 63; Colombia; DUKE; AF275175.1. ***P. austrocaledonicum* C. DC.**; MAT 530; Nueva Caledonia; GH; x. ***P. avellanum* (Miq.) C. DC.**; MAJ 109; Colombia; DUKE; EU581123.1. ***P. bartlingianum* (Miq.) C. DC.**; AL 1157; Brazil; MG; EU581125.1. ***P. basilobatum* Trel. & Yunck.**; MAJ 596; Colombia;

DUKE; AY326197.1. ***P. bavinum* C. DC.**; MAJ 392; Vietnam; DUKE; DQ882210.1. ***P. begoniicolor* Trel. & Yunck.**; TC 69629; Colombia; MO; EF056226.1. ***P. bellidifolium* Yunck.**; EJT 1430; Ecuador; MU; EF056227.1. ***P. betle* L.**; DUKE 82; Colombia; DUKE;EU581348.1. ***P. biolleyi* C. DC.**; CD 10896; Costa Rica; RSP; EU581128.1. ***P. bisasperatum* Trel.**; EJT 1054; Panama; MU; EU581129.1. ***P. blattarum* Spreng.**; CMT 11645; Puerto Rico; MO; EU581130.1. ***P. brachypodon* (Benth.) C. DC.**; MAJ 728; Colombia; DUKE; x. ***P. brachypus* Trel.**; MAJ 550; México; DUKE; EU581132.1. ***P. bradei* Yunck.**; MAJ 903; Brazil; RB; EU581133.1. ***P. breviamentum* C. DC.**; MAJ 221; Philippines; DUKE; EU581484.1. ***P. brevipedicellatum* Bornst.**; MAJ 544; Mexico; DUKE; AF275189.1. ***P. brisasense* Yunck.**; MAJ 89; Colombia; HUA; x. ***P. brownsbergense* Yunck.**; EJT 619; French Guiana; MU; EU581135.1. P. buchii Urb.; MAJ 1281; Dominican Republic; HUA; x. ***P. bullosum* C. DC.**; EJT 1405; Ecuador; MU; EU581137.1. ***P. bullulatum* M.A. Jaram.**; MAJ 55; Colombia; DUKE; AF275167.1. ***P. cabellense* C. DC.**; MAJ 87; Colombia; DUKE; AF275178.1. ***P. cabralanum* C. DC.**; MAJ 917; Brazil; RB; x. ***P. cajambrense* Trel. & Yunck.**; MAJ 768; Colombia; DUKE; AY326199.1. ***P. calcariforme* Tebbs**; MAJ 1067; Panama; HUA; x. ***P. caldense* C. DC.**; MAJ 797; Brazil; RB; EU581139.1. ***P. callosum* Ruiz & Pav.**; MAJ 1136; Bolivia; HUA; x. ***P. campanum* Yunck.**; EJT 1033; Panama; MU; EF056235.1. ***P. candollei* Sodiro**; EJT 1449; Ecuador; MU; EF056237.1. ***P. capense* L. f.**; RF 96-75; Tanzania; US; AY326200.1. ***P. caracasanum* Bredem. ex Link**; WM 1180; x; MO; EF056238.1. ***P. cararense* Trel. & Yunck.**; MAJ 601; Colombia; DUKE; AY326201.1. ***P. carautensei* E.F. Guim. & M. Carvalho-Silva**; MAJ 933; Brazil; RB; EU581146.1. ***P. carrilloanum* C. DC.**; AKM 4338; Panama; BM; x. ***P. cavendishioides* Trel. & Yunck.**; MAJ 70; Colombia; DUKE; AF275153.1. ***P. cenocladum* C. DC.**; EJT 185; Costa Rica; MU; EF056239.1. ***P. cernuum* Vell.**; MAJ 814; Brazil; RB; EU581151.1. ***P. chimonanthifolium* Kunth**; MAJ 949; Brazil; RB; x. ***P. chuarense* M.A. Jaram. & Callejas**; MAJ 721; Colombia; DUKE; AY326202.1. ***P. cihuatlanense* Bornst**.; MAJ 543; Mexico; DUKE; AF275187. ***P. cililimbum* Yunck.**; AL 1235; Brazil; MG; x. ***P. cinereum* C. DC.**; MAJ 653; Ecuador; DUKE; EU581154.1. ***P. coccoloboides* Kunth**; HSI 22879; Brazil; US; EF056243.1. ***P. cocornanum* Trel. &**

Yunck.; RC 12493; Colombia; HUA; AY326203.1. ***P. colligatispicum* Trel. & Yunck.**; MAJ 780; Colombia; DUKE; AY326204.1. ***P. collium* (Trel.) Callejas**; MAJ 1114; Panama; no voucher; x. ***P. colonense* C. DC.**; AB 753; Nicaragua; SEMO; EU581156.1. ***P. comatum* Trel.**; MAJ 1110; Panama; HUA; x. ***P. concepcionis* Trel.**; MAJ 627; Colombia; DUKE; EU581160.1. ***P. confertinodum* (Trel. & Yunck.) M.A. Jaram. & Callejas**; MAJ 54; Colombia; DUKE; AF275166.1. ***P. confusum* C. DC.**; AHL 14556; Cuba; NY; EU581162.1. ***P. consanguineum* Kunth**; EJT 546; French Guiana; MU; EU581163.1. ***P. cooperi* Yunck.**; MAJ 1069; Panama; HUA; x. ***P. corcovadensis* (Miq.) C. DC.**; MAJ 838; Brazil; RB; EU581165.1. ***P. cordatilimbum* Quisumb.**; MAJ 178; Philippines; DUKE; AY572323.1. ***P. cordilimbum* C. DC.**; MAJ 771; Colombia; DUKE; x. ***P. cordulatum* C. DC.**; BCI; Panama; no voucher; x. ***P. costatum* C. DC.**; TF 4261; x; PTBG; EU581171.1. ***P. crassinervium* Kunth**; AM 1183; Bolivia; MO; EU581172.1. ***P. cubataonum* C. DC.**; MJK 493; Brazil; SPF; EU581173.1. ***P. cumanense* Kunth**; MAJ 735; Colombia; DUKE; x. ***P. curtirachis* W.C. Burger**; EJT 995; Panama; MU; EU581175.1. ***P. curtispicum* C. DC.**; EJT 1028; Panama; MU; EU581176.1. ***P. cuspidispicum* Trel.**; AKM 5037; Panama; BM; x. ***P. daguanum* C. DC.**; MAJ 1044; Panama; HUA; x. ***P. darienense* C. DC.**; MAJ 103; Colombia; DUKE; AF275181.1. ***P. davidianum* C. DC.**; MAJ 1103; Panama; HUA; x. ***P. decurrens* C. DC.**; DSA 320; Costa Rica; MO; x. ***P. densum* Blume**; MAJ 508; Vietnam; DUKE; AY615963.1. ***P. depressibaccum* Trel.**; AL 18790; Dominican Republic; HUA; x. ***P. dichroostachyum* Trel. & Yunck.**; MAJ 1317; Colombia; CUCV; x. ***P. diguetii* C. DC.**; MAJ 546; Mexico; DUKE; x. ***P. diospyrifolium* Kunth**; MJK 431; Brazil; SPF; EU581181.1. ***P. discophorum* C. DC.**;TA 389; Colombia; HUA; x. ***P. divaricatum* G**. Mey.; EJT 1424; Ecuador; MU; EU581182.1. ***P. dolichotrichum* Yunck.**; MAJ 129; Colombia; DUKE; EU581185.1. ***P. dryadum* C. DC.**; EJT 1047; Panama; MU; EU581186.1. ***P. duartei* E.F. Guim. & M. Carvalho-Silva**; MAJ 928; Brazil; RB; EU581187.1. ***P. dumosum* Rudge**; EJT 532; French Guiana; MU; EU581188.1. ***P. entradense* Trel. & Yunck.**; EJT 1394; Ecuador; MU; EU581190.1. ***P. erectipilum* Yunck.**; MJK 762; Brazil; MG; EU581191.1. ***P. esperancanum* Yunck.**; AL 1148; Brazil; MG; x. ***P. eucalyptifolium* Rudge**; EJT 526; French Guiana; MU; EU581192.1. ***P. euryphyllum* C. DC.**; EJT 173;

Costa Rica; MU; EF056249.1. ***P. eustylum* Diels**; EJT 1400; Ecuador; MU; EU581194.1. ***P. excelsum* G. Forst**.; ROG 8494; New Zealand; AK; AF275193.1. ***P. exiguicaule* Yunck.**; MAJ 1085; Gorgona; CUVC; x. ***P. fallens* Trel.**; NM 573; Mexico; HEM; EU581195.1. ***P. filistilum* C. DC.**; MAJ 680; Ecuador; DUKE; EU581197.1. ***P. fimbriulatum* C. DC.**; EJT 115; Costa Rica; MU; EF05625.1. ***P. flagellicuspe* Trel. & Yunck.**; MAJ 65; Colombia; DUKE; AF275154.1. ***P. flavicans* C. DC.**; HSI 2102; Bolivia; USZ; x. ***P. flavidum* C. DC.**; MA 2826; Mexico; HEM; EU581203.1. ***P. flavoviride* C. DC.**; MJK 98; Brazil; SPF; EU581205.1. ***P. fonteboanum* Yunck.**; ILA 106885; Colombia; US; EF056255.1. ***P. friedrichsthalii* C. DC.**; MAJ 1057; Panama; HUA; x. ***P. frutescens* C. DC.**; MAJ 878; Brazil; RB; EU581208.1. ***P. fuligineum* Kunth**; MAJ 979; Brazil; RB; EU581209.1. ***P. garagaranum* C. DC.**; CD 10869; Costa Rica; SRP; EU581211.1. ***P. gaudichaudianum* Kunth**; MAJ 918; Brazil; RB; x. ***P. gaumeri* Trel.**; MAJ 1020; Mexico; HUA; x. ***P. gesnerioides* Callejas**; MAJ 594; Colombia; DUKE; AY326206. ***P. gibbosum* C. DC.**; EJT 170; Costa Rica; MU;EF056256.1. ***P. gigantifolium* C. DC.**; MAJ 826; Brazil; RB; x. ***P. gigas* Trel.**; EJT 1000; x; MU; EF056260.1. ***P. glabratum* Kunth**; CD 10128; x; x; x. ***P. glabrescens* (Miq.) C. DC.**; EJT 999; Costa Rica; MU; x. ***P. glanduligerum* C. DC.**; MAJ 588; Colombia; DUKE; EU581219.1. ***P. goesii* Yunck.**; MAJ 912; Brazil; RB; EU581220.1. ***P. grande* Vahl**; AB 725; Ecuador; SEMO; KM266029.1. ***P. guayranum* C. DC.**; MAJ 78; Colombia; DUKE; x. ***P. guazacapanense* Trel. & Standl.**; MA 2923; Mexico; HEM; EU581228.1. ***P. hartwegianum* (Benth.) C. DC.**; MAJ 781; Colombia; DUKE; AY326207.1. ***P. haughtii* Trel. & Yunck.**; MAJ 98; Colombia; DUKE; EU581233.1. ***P. hebetifolium* W.C. Burger**; EJT 454; Costa Rica; MU; EF056262. ***P. herzogii* C. DC.**; MAJ 1258; Bolivia; MO; x. ***P. heterophyllum* Ruiz & Pav.**; MAJ 1180; Bolivia; MO; x. ***P. hirtellipetiolum* C. DC.**; EJT 983; Panama; MU; EU581234.1. ***P. hirtulum* (C. Presl) C. DC.**; MAJ 887; Brazil; RB; x. ***P. hispidum* Sw.**; MAJ 847; Brazil; RB; EU581244.1. ***P. hoffmanseggianum* Schult.**; MAJ 891; Brazil; RB; EU581246.1. ***P. holdridgeanum* W.C. Burger**; MAJ 1052; Panama; HUA; x. ***P. hooglandii* (I. Hutton & P.S. Green) M.A. Jaram.**; ROG 8496; New Zealand; AK; AF275192.1. ***P. hostmannianum* (Miq.) C. DC.**; EJT 573; French Guiana; MU;EU581249.1. ***P.***

humistratum* Görts & K.U. Kramer**; EJT 542; French Guiana; MU; EU581252.1. ***P. hymenophyllum* Miq.**; MAJ 505; Vietnam; DUKE; AY572327.1. ***P. ilheusense* Yunck.**; MAJ 786; Brazil; RB; EU581254.1. ***P. imberbe* Trel. & Standl.**; AB 983; Honduras; SEMO; EU581255.1. ***P. immutatum* Trel.**; EJT 1590; Ecuador; MU; EU581256.1. ***P. imperiale* (Miq.) C. DC.**; MAJ 61; Colombia; DUKE; AF275176.1. ***P. inaequale* C. DC.**; MAJ 118; Colombia; DUKE; EU581259.1. ***P. insipiens* Trel. & Yunck.**; EJT 533; French Guiana; MU; EU581262.1. ***P. irazuanum* C. DC.**; RM 8049; Costa Rica; INBIO; x. ***P. juliflorum* Nees & Mart.**; MJK 257; Brazil; SPF; EU581265.1. ***P. klotzschianum* (Kunth) C. DC.**; MAJ 976; Brazil; RB; EU581266.1. ***P. kuntzei* C. DC.**; MAJ 1259; Bolivia; MO; x. ***P. lacunosum* Kunth**; EJT 443; Costa Rica; MU; EU581267.1. ***P. ladrillense* Trel.**; AKM 4193; Panama; BM; x. ***P. laevigatum* Kunth**; JS 7167; Peru; UMO; EU581269.1. ***P. laevilimbum* C. DC.**; SGB 8276; Bolivia; USZ; x. ***P. laosanum* C. DC.**; MAJ 468; Vietnam; DUKE; AY572326.1. ***P. lepturum* Kunth**; MAJ 967; Brazil; RB; EU581277.1. ***P. littorale* C. DC.**; MAJ 130; Colombia; DUKE; EU581278.1. ***P. lolot* C. DC.**; MAJ 234; Vietnam; DUKE; AY326208.1. ***P. longestylosum* C. DC.**; MAJ 1134; Bolivia; MO; x. ***P. longicaudatum* Trel. & Yunck.**; EJT 1578; Ecuador; MU; EU581281.1. ***P. longispicum* C. DC.**; MAJ 739; Colombia; DUKE; AY326209.1. ***P. longivillosum* Trel. & Yunck.**; MAJ 605; Colombia; x; x. ***P. lunulibracteatum* C. DC.**; VZ 2854; Ecuador; x; EF056267.1. ***P. luteobaccum* Trel.**; MM 23730; Dominican Republic; JBSD; x. ***P. maranyonense* Trel.**; MAJ 58; Colombia; DUKE; x. ***P. marequitense* C. DC.**; MAJ 772; Colombia; DUKE; AY326210.1. ***P. marginatum* Jacq.**; MAJ 713; Ecuador; DUKE; AY326211. ***P. marsupiiferum* Trel.**; EJT 1431; Ecuador; MU; EF056269.1. ***P. martensianum* C. DC.**; AB 746; Nicaragua; SEMO; EU581294.1. ***P. massiei* C. DC.**; MAJ 478; Vietnam; DUKE; EU581295.1. ***P. maxonii* C. DC.**; EJT 370; Costa Rica; MU; EF056270.1. ***P. melanocladum* C. DC.**; AKM 4382; Panama; BM; x. ***P. melastomoides* Schltdl. & Cham.**; MN 26814; Mexico; XAL; EU581297.1. ***P. methysticum* G. Forst.**; AE 16; x; NTBG; AF275194.1. ***P. mexiae* Trel. & Yunck.**; EJT 1398; Ecuador; MU; EU581299.1. ***P. michelianum* C. DC.**; MAJ 540; Mexico; DUKE; x. ***P. mollicomum* Kunth**; MAJ 795; Brazil; RB; EU581301.1. ***P. mosenii* C. DC.**; MAJ 876; Brazil; RB; EU581302.1. ***P.

mourai **Yunck.**; MAJ 914; Brazil; RB; EU581303.1. ***P. muelleri*** **C. DC.**; MAJ 541; México; DUKE; EU581304.1. ***P. multiplinervium*** **C. DC.**; MAJ 1043; Panama; HUA; x. ***P. munchanum*** **C. DC.**; MAJ 120; Colombia; DUKE; AF275164.1. ***P. napo-pastazanum*** **Trel. & Yunck.**; EJT 1589; Ecuador; MU; EU581308.1. ***P. neesianum*** **C. DC.**; MAJ1030; Mexico; HUA; x. ***P. nicoyanum*** **C. DC.**; UC 1634; Costa Rica; HUA; EU581312.1. ***P. nigro-granulatum*** **Trel.**; MAJ 1173; Bolivia; MO; x. ***P. nitidum*** **Sw.**; AR 2312; México; XAL; EU581314.1. ***P. nobile*** **C. DC.**; TC 60527; Venezuela; MO; EU581315.1. ***P. novogalicianum*** **Bornst**.; MAJ 549; Mexico; DUKE; EU581316.1. ***P. novo-granatense*** **C. DC.**; MAJ 80; Colombia; DUKE; x. ***P. nudifolium*** **C. DC.**; EJT 973; Panama; MU; EU581321.1. ***P. obliquum*** **Ruiz & Pav.**; EJT 1022; Panama; MU; EU581323.1. ***P. obovatum*** **Vahl**; MAJ 641; Colombia; DUKE; AY326212.1. ***P. obtusilimbum*** **C. DC.**; EJT 512; x; MU; EU581328.1. ***P. orthostachyum*** **Kunth**; MAJ 1241; Bolivia; HUA; x. ***P. otophorum*** **C. DC.**; EJT 981; Panama; MU; EU581329.1. ***P. ottoniaefolium*** **C. DC.**; MAJ 703; Colombia; DUKE; x. ***P. ovatum*** **Vahl**; NJsn; Trinidad & Tobago; no voucher; x. ***P. oviedoi*** **Urb.**; MAJ 1286; Dominican Republic; HUA; x. ***P. oxystachyum*** **C. DC.**; MAJ 140; Colombia; DUKE; AF275152.1. ***P. paramaribense*** **C. DC.**; EJT 544; French Guiana; MU; EU581332.1. ***P. partiticuspe*** **Trel.**; MAJ 1093; Panama; HUA; x. ***P. pedunculatum*** **C. DC.**; MAJ 597; Colombia; DUKE; EU581333.1. ***P. peltatum*** **L.**; MAJ 1074; Panama; HUA; x. ***P. peracuminatum*** **C. DC.**;MAJ 1086; Panama; HUA; x. ***P. perareolatum*** **C. DC.**; AlG 74657; Peru; MO; EF0566279.1. ***P. perbrevicaule*** **Yunck.**; MAJ 1044; Panama; HUA; x. ***P. perlasense*** **Yunck.**; BCI; Panama; no voucher; x. ***P. perpusillum*** **Callejas**; MAJ 699; Ecuador; DUKE; AY326215.1. ***P. perscabrifolium*** **Yunck.**; MAJ 1249; Bolivia; MO; x. ***P. phytolaccifolium*** **Opiz**; MAJ 599; Colombia; DUKE; AY326216.1. ***P. pilibracteum*** **Trel. & Yunck.**; MAJ 746; Colombia; DUKE; AY768829.1. ***P. piluliferum*** **Kunth**; EJT 1411; Ecuador; MU; EU581340.1. ***P. jacquemontianum*** **Kunth**; AB 700; Honduras; SEMO; EU581260.1. ***P. tomas-albertoi*** **Trel. & Yunck.**; Rcsn; Colombia; no voucher; x. ***P. piscatorum*** **Trel. & Yunck.**; MJK 758; Brazil; MG; U581342. ***P. pittieri*** **C. DC.**; AKM 5303; Panama; BM; x. ***P. preaesagium*** **Trel. & Yunck.**; MAJ 68; Colombia; DUKE; EU581347.1. ***P. premnospicum*** **Tebbs**; EJT 1030; Panama; MU; EU581349.1. ***P.***

***prismaticum* C. DC.**; DSA 322; Costa Rica; MO; x. ***P. pseudobumbratum* C. DC.**; DSA 327; Costa Rica; MO; x. ***P. pseudofuligineum* C. DC.**; AB 710; Honduras; SEMO; EU581350.1. ***P. pseudogrande* Yunck.**; MAJ 1187; Panama; HUA; x. ***P. pseudolanceifolium* Trel.**; KHAN 1297; Costa Rica; MO; EU581351.1. ***P. pseudolindenii* C. DC.**; AB 711; Honduras; SEMO; EU581353.1. ***P. pseudonobile* C. DC.**; BB 3521; Ecuador; MO; EF056281.1. ***P. pseudopothifolium* C. DC.**; MAJ 943; Brazil; RB; EU581356.1. ***P. psilorhachis* C. DC.**; MAJ 1013; Mexico; HUA; x. ***P. pterocladum* C. DC.**; AM 1079; Bolivia; MO; EU581359.1. ***P. pubescens* Vahl**; BCI; Panama; no voucher; x. ***P. pubistipulum* C. DC.**; EJT 976; Panama; MU; EU581361.1. ***P. pulchrum* C. DC.**; AlG 65422; x; x; x. ***P. reticulatum* L.**; MAJ 722; Colombia; DUKE; x. ***P. retrofractum* Vahl**; MAJ 395; Vietnam; DUKE; EU581602.1. ***P. richardiifolium* Kunth**; MAJ 925; Brazil; RB; EU581368.1. ***P. rio-docense* E.F.Guim. & Carv.-Silva**; GV 430; Brazil; RB; EU594345.1. ***P. riparense* C. DC.**; EJT 339; Costa Rica; MU; EU581603.1. ***P. rivinoides* Kunth**; MAJ 973; Brazil; RB; x. ***P. robustipedunculum* Yunck.**; MAJ 871; Brazil; RB; EU581373.1. ***P. rugosum* Lam.**; AV 3727; Haiti; JBSD; x. ***P. rusbyi* C. DC.**; AM 1216; Bolivia; MO; EU581375. ***P. sabaletasanum* Trel. & Yunck.**; MAJ 623; Colombia; DUKE; AY326217.1. ***P. sagittifolium* C. DC.**; EJT 320; Costa Rica; MU; EF056285.1. ***P. samanense* Urb.**; MAJ 1275; Dominican Republic; HUA; x. ***P. sampaioi* Yunck.**; MAJ 916; Brazil; RB; EU581378.1. ***P. sancti-felicis* Trel.**; AR 2429; Mexico; XAL; EU581379.1. ***P. sanctum* (Miq.) Schltdl. ex C. DC.**; AB 744; Nicaragua; SEMO; EU581382.1. ***P. sasaimanum* Yunck.**; EJT 1055; Panama; MU; EF056286.1. ***P. scalpens* Trel.**; RM 8052; Costa Rica; INBIO; x. ***P. schiedeanum* Steud.**; BCI; Panama; no voucher; x. ***P. schuppii* A.H. Gentry**; MAJ 687; Ecuador; DUKE; AY326218.1. ***P. schwackei* C. DC.**; MJK 732; Brazil; MG; EU581387.1. ***P. scutifolium* Yunck.**; MJK 281; Brazil; SPF; EU581389.1. ***P. scutilimbum* C. DC.**; EJT 1438; Ecuador; MU; EU581390.1. ***P. secundum* Ruiz & Pav.**; MAJ 1242; Bolivia; MO; x. ***P. seducentifolium* Trel.**; MAJ 1120; Panama; HUA; x. ***P. silvarum* C. DC.**; MAJ 1182; Bolivia; MO; x. ***P. silvivagum* C. DC.**; DSA 315; Costa Rica; MO; x. ***P. sociorum* Trel. & Yunck.**; MAJ 1078; Panama; HUA; x. ***P. solmsianum* C. DC.**; MAJ 881; Brazil; RB; EU581392.1. ***P. sorsogonum* C. DC. Ex**

Quisumb; MAJ 185; Philippines; DUKE; AY572320.1. ***P. sphaerocarpum* C. DC. ex C. Wright**; JRA 18913; Cuba; FLAS; EU581397.1. ***P. spoliatum* Trel. & Yunck.**; MAJ 60; Colombia; DUKE; AF275179.1. ***P. sprengelianum* C. DC.**; MAJ 837; Brazil; RB; EU581398.1. ***P. sternii* Yunck.**; EJT 1393; Ecuador; MU; EU581399.1. ***P. stiliferum* Yunck.**; EJT 1631; Ecuador; MU; EU581400.1. ***P. stipulaceum* Opiz**; MAJ 551; México; DUKE; EU581401.1. ***P. subflavum* C. DC.**; BRR 1736; Colombia; HUA; EU581402.1. ***P. subglabribracteatum* C. DC.**; MAJ 747; Colombia; DUKE; AY326220.1. ***P. subpedale* Trel. & Yunck.**; MAJ 57; Colombia; DUKE; AF275161.1. ***P. subscutatum* (Miq.) C. DC.**; EJT1 604; Ecuador; MU; EU581406.1. ***P. subsessilifolium* C. DC.**; EJT 1003; Panama; MU; EU581329.1. ***P. subsilvulanum* C. DC.**; RC1 3867; Bolivia; HUA; x. ***P. tabanicidum* Trel.**; RM 8111; Costa Rica; INBIO; x. ***P. tardans* Trel.**; EJT 1056; Panama; MU; EF056290.1. ***P. tectoniifolium* Kunth**; EJT 547; French Guiana; MU; EU581408.1. ***P. terryae* Standl.**; MAJ 605; Colombia; DUKE; AY326221.1. ***P. thomasii* Tebbs**; EJT 1038; Panama; MU; EU581409.1. ***P. tonduzii* C. DC.**; DSA 314; Costa Rica; MO; x. ***P. toppingii* C. DC.**; MAJ 186; Philippines; DUKE; AY572322.1. ***P. trianae* C. DC.**; MAJ 648; Colombia; DUKE; x. ***P. trichogynum* C. DC.**; MAJ 1199; Bolivia; MO; x. ***P. trichoneuron* (Miq.) C. DC.**; EJT 549; French Guiana; MU; EU581414.1. ***P. tricuspe* (Miq.) C. DC.**; AG 41; Colombia; CUCV; AY326224. ***P. trigonum* C. DC.**; CD 19874; x; x; x. ***P. truncatum* Vell.**; MAJ 896; Brazil; RB; x. ***P. tuberculatum* Jacq.**; MAJ 710; Ecuador; DUKE; AY326225.1. ***P. tuerckheimii* C. DC.**; AB 941; Honduras; SEMO; EU581427.1. ***P. turbense* Trel.**; MAJ 1105; Panama; UMO; x. ***P. ubatubense* Callejas**; CC 2; x; DUKE; AF275182.1. ***P. ucalyptiphyllum* C.DC.**; MAJ 972; Brazil; RB; EU581193.1. ***P. umbellatum* L.**; MAJ 35; Colombia; DUKE; EU581433.1. ***P. umbricola* C. DC.**; DSA 305; Costa Rica; MO; x. ***P. umbriculum* M.A. Jaram. & Callejas**; MAJ 602; Colombia; DUKE; AY326226.1. ***P. unispicatum* Callejas**; RC 11854; Colombia; HUA; AY326227.1. ***P. urophyllum* C. DC.**; DSA 308; Costa Rica; MO; x. ***P. urostachyum* Hemsl.**; AB 757; Nicaragua; SEMO; EU581437.1. ***P. varablancanum* Trel.**; MAJ 1064; Panama; HUA; x. ***P. vellosoi* Yunck.**; MAJ 872; Brazil; RB; EU581439.1. ***P. vicosanum* Yunck.**; MAJ 809; Brazil; RB; EU581440.1. ***P. villiramulum* C. DC.**; AB 947; Honduras; SEMO;

EU581441.1. ***P. villosum* C. DC.**; MAJ 667; Ecuador; DUKE; AY326228.1. ***P. vitaceum* Yunck.**; RV 14005; Brazil; MG; x. ***P. wachenheimii* Trel.**; EJT 574; French Guiana; MU; EU581443.1. ***P. xanthostachyum* C. DC.**; AB 732; Nicaragua; SEMO; EU581445.1. ***P. xylosteoides* (Kunth) Steud.**; MJK 262; Brazil; SPF; EU581447.1. ***P. yanaconasense* Trel. & Yunck.**; MAJ 774; Colombia; DUKE; AY326229.1. ***P. yessupianum* Trel.**; MAJ 1169; Bolivia; HUA; x. ***P. yucatanense* C. DC.**; MAJ 1031; Mexico; HUA; x.

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APPENDIX B. List of collection vouchers for species used in morphological analysis. ***Taxon; number v***oucher specimen.

Piper bartlingianum; NY01421611, NY01421627, U0115535. ***P. alatabaccum***; NY01421072, K000324115, NY01421072, NY01421074. ***P. piscatorum;*** NY01488514, NY01488518, NY00452751. ***P. ovatum;*** G00203537, NY00557096, NY00557108. ***P. anisum;*** NY00555803, U0005553, U0005554. ***P. aulacospermum;*** U0119486, U0119491, U0119492. ***P. orthostachyun;*** B1058083. ***P. samanense;*** B10294738, NY01373169, NY01373170. ***P. oviedoi;*** G00169957, NY01373118, NY01373121. ***P. sphaerocarpum;*** B10377301, G00169988, NY01373182. ***P. yucatanense;*** F1808389, K000323886, P00614505. ***P. psilorhachis;*** B10375231, F2285678. ***P. pseudolindenii;*** CICY30085, F2181992, K000323801. ***P. melastomoides;*** K000323657, K000323658. ***P. neesianum;*** CICY30241, F894327, MO953817. ***P. martensianum;*** P00614546, P02026208, P02026440. ***P. michelianum;*** G00169955, K000323838. ***P. stipulaceum***; Haenke F., Hinton MO11372, K000323670. ***P. abalienatum;*** MICH1111996. ***P. amalago;*** NY00826609, NY01372193, NY00555660. ***P. reticulatum;*** NY00557211, F2211808, MO5758981. ***P. laevigatum;*** P00614504, NY00235439, NY00756300. ***P. piluliferum;*** F1543063, GDC014772, P01645936. ***P. scutilimbum;*** US00107045. ***P. urophyllum;*** MO5798529, P1796063. ***P. xylosteoides;*** B101001788. ***P. glanduligerum;*** GDC014707. ***P. grande;*** F589194, F652727. ***P. aequale;*** F770233, F1892968, K000324148. ***P. perlasense;*** MO1590797. ***P. asymmetricum;*** F2105716, F1642096. ***P. carrilloanum;*** F2128193. ***P. rivinoides;*** K000324020. ***P. solmsianum;*** NY01801092. ***P. lepturum;*** GDC014741, NY00556814. ***P. pseudobumbratum;*** MO04584643. ***P. umbellatum;*** NY00993578, G00206111. ***P. multiplinervium;*** F1990292, NY00756331. ***P. schwackei;*** NY01021344, NY01360338, NY01360339. ***P. marginatum;*** NY00957512, F1897131, NY00826616. ***P. tuberculatum;*** US00107620, B-W00676 -02 0, F21955480. ***P. cumanense;*** MAJ767. ***P. perareolatum;*** F2178620. ***P. pseudonobile;*** B10250465. ***P. sagittifolium;*** F1715426. ***P. tardans;*** MO1171074, US00107198. ***P. melanocladum;*** F1712979, F1947719, MO05033359. ***P. obovatum;*** US1176978. ***P. tomas-albertoi;*** US00107179. ***P.***

richardiifolium*;* NY00557214, NY00557220. ***P. truncatum****;* NY00557306, NY00557318, NY00557307. ***P. aereum;*** ILL00007885, US00107502, ILL00007884. ***P. sabaletasanum****;* US1856436. ***P. sociorum****;* US00107113, U0005629. ***P. maxonii****;* MO1190793, NY00250940. ***P. longispicum****;* F578457, NY00251448. ***P. yanaconasense****;* F1286580. ***P. nudifolium****;* MO1046542. ***P. trigonum****;* F1808781, K000323841, K000324378. ***P. subpedale****;* F1267028, US00107166. ***P. turbense****;* NY00283826, U0016280. ***P. confertinodum****;* MAJ733, U0016242. ***P. longivillosum****;* US00106620. ***P. terryae****;* F1034541. ***P. nigrogranulatum****;* NY002515531. ***P. prismaticum****;* MO05033396. ***P. phytolaccifolium****;* F1796060. ***P. munchanum****;* K000324307. ***P. luteobaccum****;* B100250513, US00106614. ***P. adenandrum****;* NY01421155, NY01421154. ***P. longestylosum****;* F685370, F1756476. ***P. heterophyllum****;* GDC0147281. ***P. vicosanum****;* F1518264. ***P. amplum****;* ASU0058753. ***P. pseudofuligineum****; F1873073.*