Floral cost vs. floral display: Insights from the megadiverse Myrtales suggest that energetically expensive floral parts are less phylogenetically constrained[1]

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- *Premise of the study:* Floral display describes the effect of flower size combined with the number of flowers per inflorescence. There is strong evidence that a floral-display trade-off operates under energetic constraint, with few-flowered inflorescences likely to have larger flowers than many-flowered inflorescences. Flower size can be estimated by different variables; thus, we propose that the variable for flower size that is most highly (negatively) correlated with the number of flowers per inflorescence will also be the best estimate of floral cost. Ranking the correlation with the phylogenetic signal of the variable can provide additional insight into the evolution of floral display.
- *Methods:* The Myrtales were chosen as a model order based on age, worldwide distribution, and diversity of reproductive strategies. Ninety-nine species representing all families and one quarter of generic diversity across its geographic and ecological range were sampled to reconstruct a phylogeny based on *rbcL* and *ndhF* sequences. Correlation coefficients were calculated for flower size variables vs. the number of flowers per inflorescence. Phylogenetic signal was measured for all variables and for floral display.
- *Key results:* Flowers per inflorescence showed significant negative correlation with the following flower size variables (weakest to strongest): filament length < anther size < flower depth < flower diameter. As the correlation of each character with number of flowers per inflorescence rose (suggesting increased cost), the values for phylogenetic signal diminished (suggesting less constraint).
- *Conclusions:* We conclude that energetically costly floral characters appear to be less phylogenetically constrained, while low-cost floral characters maintain higher levels of phylogenetic inertia.

Key words: evolution; flower diameter; floral display; Myrtales; phylogenetic signal; trade-off.

Evolutionarily, form and function are intimately related. In angiosperms, floral morphology has been considered to affect the mode and efficiency of pollination and reproduction since Sprengel (1793), whose work was promoted by Darwin (1877) and summarized in biological classics such as the one by Faegri and van der Pijl (1979). Among the numerous floral characters that exist, flower size is considered one of the most important aspects of floral attraction (for a recent review, see Willmer, 2011).

Flower size affects floral visibility and is associated with efficiency of pollinator selection (Kettle et al., 2011) and sexual dimorphism (Delph, 1996). Large flowers usually offer greater rewards than small flowers and are better equipped to withstand the physical stresses caused by large pollinators; small animals visiting large flowers may not act as efficient pollinators unless they are specifically adapted to its morphological idiosyncrasies (e.g., *Callistemon*, Paton, 1993). Small flowers, on the other hand, are adapted to the bodies and needs of small animals and may offer rewards that are either physically inaccessible to larger animals or are too small to attract them (Kettle et al., 2011). During angiosperm evolution, interactions between plants and their pollinators have resulted in selective pressures on flower size, producing enormous variation of this feature, from the minuscule flowers of *Wolffia* (Araceae) that measure only 0.25 mm in diameter (Bernard et al., 1990) to giant *Rafflesia arnoldii* (Rafflesiaceae) flowers that are ca. 4000 times larger, measuring 1000 mm (Beaman et al., 1988).

Within a species, flower size is usually less variable than that of other plant parts (such as leaves and fruits) due to the stabilizing effect of fine-tuned plant–pollinator coevolution (Worley and Barrett, 2000). However, within a phylogeny, flower size may vary widely even among close congeners (Worley and

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Barrett, 2000; Davis et al., 2008). The capability for adaptive shifts in flower size may be important when there is a change in pollinator availability. Such a change can bring about new selective pressures that could alter floral structure and/or lead to a new floral size phenotype (reviewed by Fenster et al., 2004). Floral structure adaptability may have played a significant part in angiosperm evolution and in maintaining present day high angiosperm diversity (Fontaine et al., 2006; Crepet and Niklas, 2009; Kay and Sargent, 2009).

Many macroecological or community studies include a variable called flower size, which usually encompasses the energetic investment in individual floral production. Such studies almost invariably use flower diameter as a surrogate for flower size (e.g., Stanton et al., 1991; Meagher, 1992, 1994; Schemske and Agren, 1995; Carroll and Delph, 1996; Conner and Rush, 1996; Delph, 1996; Schemske et al., 1996; Elle, 1998; Morgan, 1998; Worley and Barrett, 2000, 2001; Thompson et al., 2002; Sargent et al., 2007; Kettle et al., 2011; see also references listed by Worley and Barrett, 2000). Other surrogates, such as floral depth, are rarely used (but see Delph and Herlihy, 2012), although it is obvious that these are also important in some floral strategies.

Floral display, which is a term coined for flower size combined with the number of flowers per inflorescence, determines how pollinators perceive an inflorescence (de Jong and Klinkhamer, 1994; Conner and Rush, 1996; Harder and Barrett, 1996; Worley and Barrett, 2000). Angiosperm floral displays can influence pollen transfer, reproductive success, and the general level of fitness of a species (Wyatt, 1982; de Jong and Klinkhamer, 1994; Conner and Rush, 1996; Harder and Barrett, 1996; Ishii et al., 2008; Kettle et al., 2011). In studies concerned with floral strategy and pollinator response, the term floral display often refers to the mean number of flowers open per day in an inflorescence, but if energetic investment is the focus, then total number of flowers per inflorescence, not just those on a daily basis, rather than flower size is considered more appropriate (Sargent et al., 2007).

In floral display, there is often a trade-off between flower size and the number of flowers per inflorescence (Stanton et al., 1991; Meagher, 1992, 1994; Schemske and Agren, 1995; Carroll and Delph, 1996; Delph, 1996; Schemske et al., 1996; Elle, 1998; Morgan, 1998; Worley and Barrett, 2000; Sargent et al., 2007; Kettle et al., 2011). Floral display trade-off (henceforward FDT-O) means that a species produces fewer flowers per inflorescence as flower size increases (Morgan, 1993; Sakai, 2000; see Fig. 1). Phylogenetic load (i.e., the phylogenetic distance between taxa of a given analyzed group) and time of divergence influences the degree of correlation between floral size and number of flowers per inflorescence (Sargent et al., 2007). This trade-off between size and number is ecologically important, as it leads to a more equitable distribution of pollinator types due to floral display variability in the ecosystem bringing advantages for both plants and pollinators. Worley and Barrett (2000) criticized FDT-O theory supported only by empirical evidence and stated it may not be universal, but noted it does seem to be particularly significant in large, highly diverse families.

Several studies (Cohen and Dukas, 1990; Harder and Barrett, 1995, 1996; Worley and Barrett, 2000; Caruso, 2004) have argued that energetic constraint is responsible for FDT-O: plant resources are often too limited to invest simultaneously both in large and numerous flowers. Floral cost is strongly influenced by flower size (reviewed by Willmer, 2011). Other factors such as nectar production (Harder and Cruzan, 1990), UV reflection

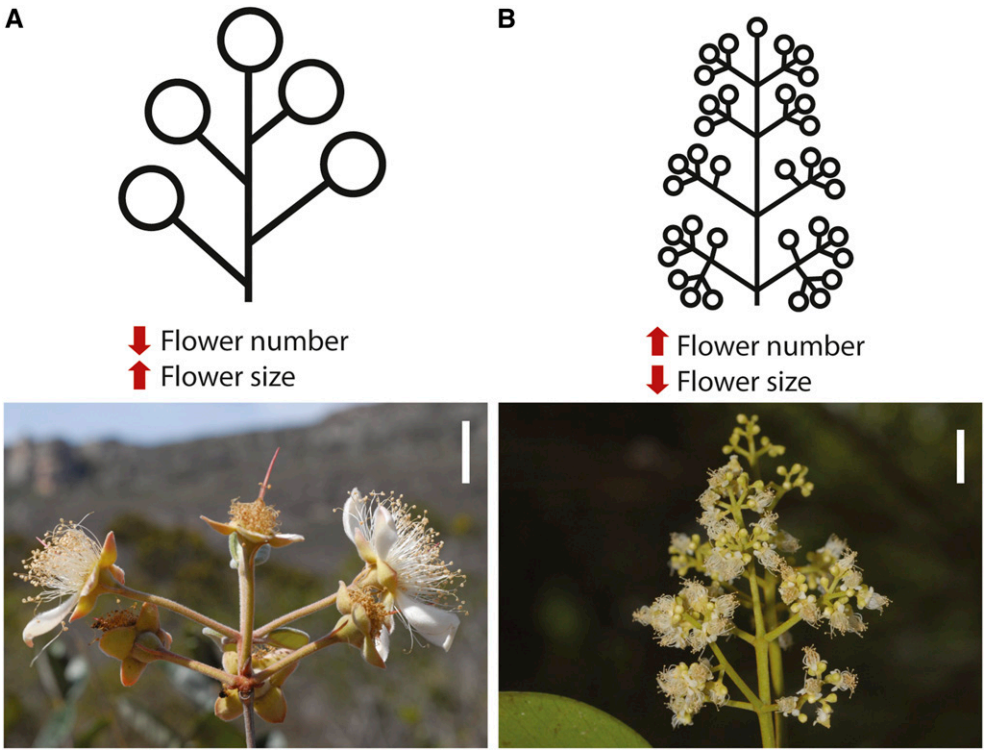

Fig. 1. Example of floral display trade-off in Myrtaceae. (A) *Eugenia azurensis*, display with a few large flowers per inflorescence. (B) *Marlierea laevigata*, display with a high number of small flowers. Scale = 10 mm. Image rights: Eve J. Lucas.

(Guldberg and Atsatt, 1975), sexual dimorphism (Delph, 1996), and even protection against predators when pollinators and predators select for different floral sizes (Galen and Cuba, 2001) are ultimately also directly related to flower size. As different flower strategies are observed, immediately obvious is that flower biomass can be allocated in many different ways, such as to flower diameter (Fig. 2, A and B), floral depth (Fig. 2, C and D) or to the androecium (Fig. 2, E and F), leading to the interesting question of the best way to estimate flower size (itself a proxy for energetic cost per flower).

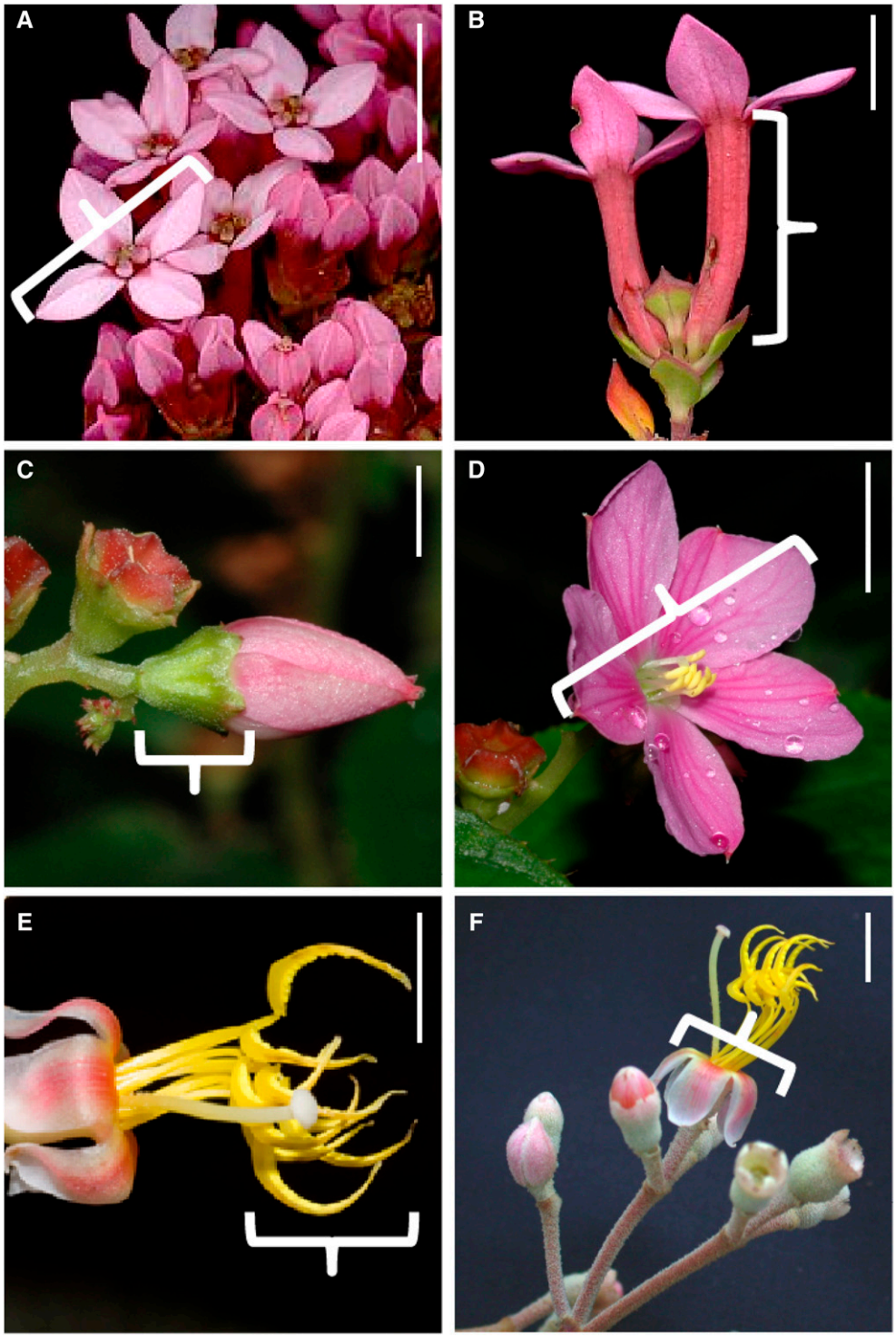

Fig. 2. Variability in biomass allocation strategies in flowers of Myrtales. (A, B) *Brachysiphon acutus* (Penaeaceae), high investment in floral depth, when compared with flower diameter. (C, D) *Calvoa orientalis* (Melastomataceae), high investment in diameter of the corolla, when compared with flower depth. (E, F) *Miconia dodecandra* (Melastomataceae): high investment in stamens and anthers. Scale = 5 mm. Image rights: (A, B) Jan De Laet; (C, D) Peter Swart; (E) Fabian A. Michelangeli, and (F) Rolando Pérez.

The existence of a strong body of evidence that FDT-O is a real phenomenon offers an opportunity to compare different floral size variables and to calculate how strongly they are correlated with flowers per inflorescence. The underlying hypothesis of our study is that if FDT-O is due to a constraint on the total energy allocated to the inflorescence, then the strengths of the negative correlations between number of flowers and the various floral size variables should reflect the energetic cost associated with each of them. Furthermore, associating the inferred cost of each floral size variable with its phylogenetic signal as estimated using the lambda index sensu Pagel (1999) could provide an insight into how phylogenetic constraint and energetic cost have interacted over evolutionary time. We asked: Does the strength of evidence for FDT-O (i.e., magnitude of correlation coefficient) depend on the variable used to represent flower size? What is the relationship between the strength of evidence for FDT-O and the level of phylogenetic constraint on a flower size variable?

MATERIALS AND METHODS

***Study group*—**The phylogenetically well-resolved rosid order Myrtales was chosen as a model based on its large size (ca. 11 000 species in 380 genera; APG III, 2009), great age (ca. 110 Myr; Sytsma et al., 2004), worldwide geographic distribution, and especially, on its diversity of flowering strategies. Species were chosen based on the simultaneous availability of suitable DNA sequence data—*rbcL* and *ndhF* chloroplast regions (GenBank consulted in February 2012)—since these two regions are the most commonly available for Myrtales, therefore maximizing sample size. Ninety-nine species, from nine families (sensu APG III, 2009; 100% of Myrtales families) and 91 genera (22.8% of Myrtales genera) from across the geographic and ecological range of the order were identified. The species list covered a wide variety of floral sizes and pollination strategies (Raven, 1979; Renner, 1984, 1989; Lughadha and Proença, 1996; Litt and Stevenson, 2003; Schonenberger and Conti, 2003; Fleming et al., 2009). Because most of the published sequences were from taxonomic studies, it is expected that authors maximized taxonomic diversity in their samples.

***Phylogenetic tree*—**A maximum likelihood tree based on the general time reversible model (Nei and Kumar, 2000) was built using the program MEGA v. 5.1 (Tamura et al., 2011). Sequences were aligned using the program Clustal X (Larkin et al., 2007), and alignment was straightforward. Initial tree(s) for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. The analysis involved 102 nucleotide sequences. Codon positions included were 1st+2nd+3rd+noncoding. Positions containing gaps and missing data were eliminated. There were a total of 1007 positions in the final data set. *Byttneria aculeata* Jacq., *Tilia americana* L. (both Malvales), and *Tropaeolum majus* L. (Brassicales) were the outgroup species; Brassicales and Malvales are both part of the rosids 2 clade to which the Myrtales belongs (APG III, 2009). GenBank accession numbers are listed in Appendix 1.

***Measuring flower cost surrogates*—**Four floral dimensions were evaluated: (1) average floral diameter; (2) average floral depth (length of the corolla or floral tube); (3) average longest anther axis; and (4) average longest filament length. These floral dimensions were chosen because they are quantitative, variable, easily available in the literature or herbarium, and affected by all floral parts: calyx (1), corolla (1), gynoecium (2), and androecium (3 and 4); only the corolla and androecium can be considered distantly developmentally related because petals are modified stamens. Floral diameter as treated here is an inclusive term that allowed us to measure apetalous flowers with rudimentary sepals, apetalous flowers with showy sepals to flowers with well-developed calyces and corollas. Floral diameter was measured as two times the petal length (or sepal length for apetalous species) plus the diameter of the hypanthium at the torus, unless the species had a single petal, in which case it was measured as hypanthium diameter plus length of the single petal. For zygomorphic species, the widest point or longest petal was considered. For apetalous species, we used sepal length. Floral depth was measured from base to torus and included the spur if present. Although nectar volume is a desirable character to include, it is unknown for most species and is only measurable in the field; however, floral depth associated with number of flowers per inflorescence is a good predictor and strongly correlated with nectar volume (Harder and Cruzan, 1990). Style length, although easy to measure, was not used because of its usually low biomass in Myrtales and strong relationship with breeding system. Filament length, although sometimes also related to breeding system, can also play a role in pollinator attraction in Myrtales, especially when considering bottle-brush flowers. Average number of flowers per inflorescence was determined by counting both buds and open flowers (potential number of flowers rather than open flowers). Floral display was measured as number of flowers per inflorescence times flower diameter.

Measurements were obtained from the taxonomic literature or from herbarium specimens: the Universidade de Brasília (UB), the Jardim Botânico do Rio de Janeiro (RB), and the Royal Botanic Gardens, Kew (K) were consulted personally, and the Missouri Botanical Garden (MO), the University of Texas (TEX), and the Arizona State University (ASU) herbaria were consulted online. The average for each floral character was calculated as the mean of five specimens chosen in each herbarium to represent the extremes for each character (i.e., extreme morphotypes for size of floral parts in the available sample) that were in adequate conditions to be measured. In the case of literature reports, average values are the midpoints between minimum and maximum values. Literature measurements were also compared with those obtained in herbaria. Analyzed herbaria specimens and literature used to cross-check all measurements are available in Appendices S1 and S2 (see Supplemental Data with the online version of this article), respectively.

***Correlation coefficients and phylogenetic signal calculation*—**Flower diameter (the most commonly used estimate of flower size) was tested for correlation with each the other three estimates of flower size. Trade-offs between each of these four characters and the average number of flowers per inflorescence was tested first by using Pearson's correlation and then phylogenetically independent contrasts (PICs) both available in the program R version 3.1.2, the first in the standard software and the second in the package APE (R Development Core Team, 2012). Phylogenetic signal (calculated using the lambda index) was applied to our ad hoc phylogenetic tree to estimate floral display, number of flowers, and characters relating to flower size change over evolutionary time. *Lambda* index varies between 0 (complete absence of phylogenetic signal, i.e., maximum lability) and 1 (maximum phylogenetic signal, i.e., high phylogenetic inertia; Pagel, 1999). Phylogenetic signal was calculated using the statistical package Geiger in R version 3.1.2.

RESULTS

***Phylogenetic tree results*—**The phylogenetic tree produced (Fig. 3) was congruent with other order-level phylogenies of Myrtales (Conti et al., 1996; Conti et al., 2002; Sytsma et al., 2004; Rutschmann et al., 2007) using the same or additional markers (Appendix 1). Combretaceae emerged as sister group to the rest of the Myrtales (agreeing with APG III, 2009) and not sister to the Lythraceae + Onagraceae clade as suggested by Conti et al. (1997); the bootstrap value (<50) found in APG III (2009) and in this study makes its position controversial.

***Correlation between different flower size surrogates and number of flowers per inflorescence*—**The number of flowers per inflorescence varied between 1 and <80, with a mean of 22 ($\sigma = 25$). Floral diameter varied from 1.5 to 85 mm, with a mean of 17 mm ($\sigma = 16$). Floral depth varied between 0.2 and 82.5 mm, with a mean of 11 mm ($\sigma = 14$). Filament length varied between 0.3 and 21 mm, with a mean value of 6.5 mm ($\sigma = 5$). Raw data for each species (number of flowers per inflorescence, flower diameter, flower depth, anther length, and filament length) are given in online Appendix S3.

The number of flowers per inflorescence was negatively correlated (Pearson's correlation) with all flower size surrogates;

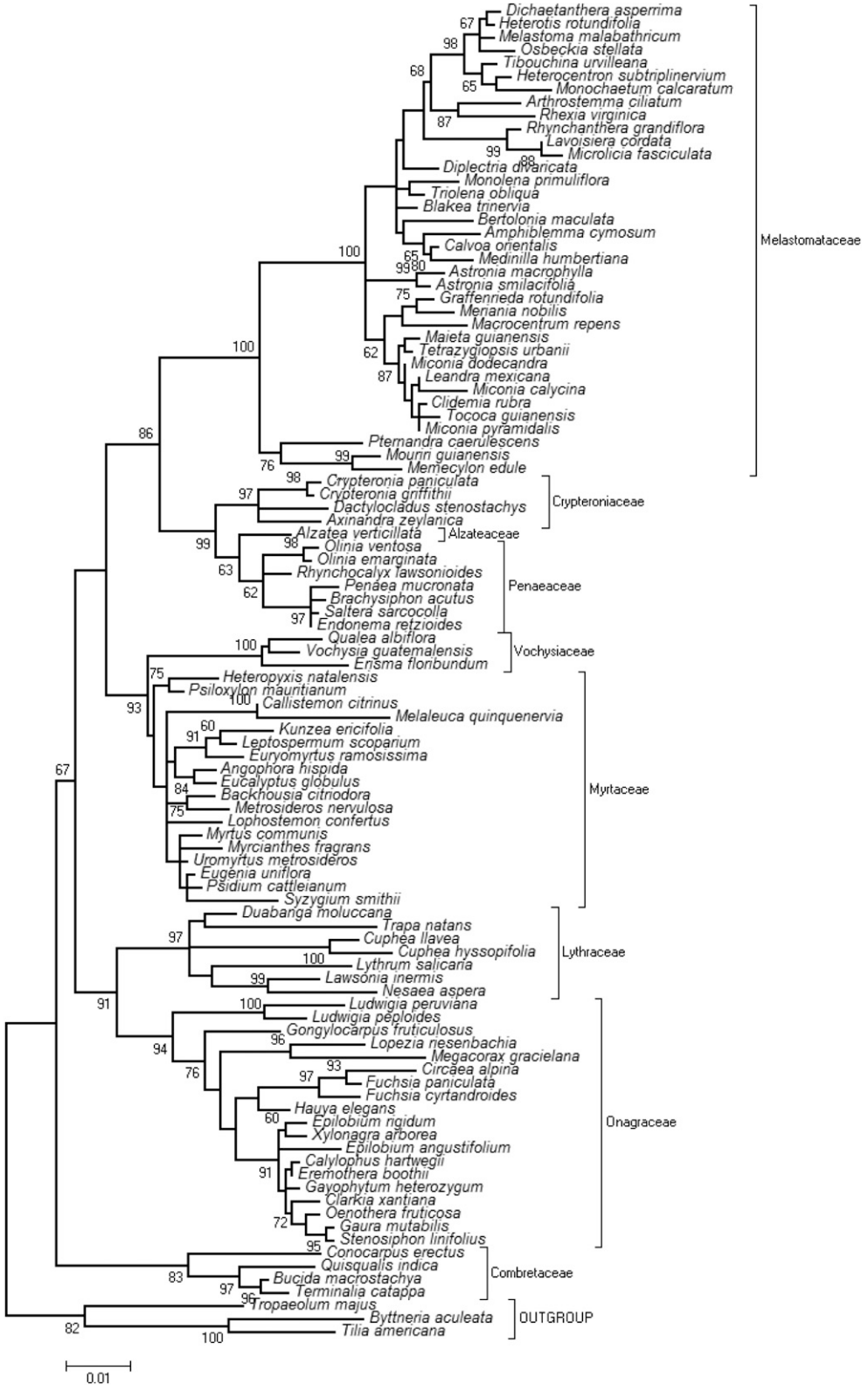

Fig. 3. Phylogenetic tree supporting this research. Tree was built in MEGA 5.1 using *rcbL* and *ndhF* of 102 nucleotide sequences. Bootstrap values below 50 are hidden. For more information, see Methods, Phylogenetic tree section.

correlations were significant for flower diameter, anther size, and floral depth (Table 1); for filament length, the correlation was close to significant ($P = 0.0554$). However, when "bottle-brush" strategy species (identified in Appendix S3) were removed from the sample, the correlations were significant for all four characters (Table 1). Bottle-brush species have many flowers

TABLE 1. Pearson and phylogenetic independent contrast correlation coefficients between the average number of flowers per inflorescence and flower size in order Myrtales, as estimated by four surrogates for floral size. Characters are ordered from the most negative to least negative correlation. Values of *P* and lambda for phylogenetic signal are also presented.

Floral size surrogates	Pearson correlation (vs. no. of flowers/ inflorescence) Correlation coefficient (*P*)	Phylogenetic independent contrast (vs. no. of flowers per inflorescence) Correlation coefficient (*P*)	Lambda
Corolla diameter	–0.370 (0.261 × 10^{-3})	–0.312 (0.107 × 10^{-2})	0.33
Floral depth	–0.236 (0.024)	–0.229 (0.021)	0.571
Anther longest axis	–0.206 (0.049)	–0.178 (0.044)	0.819
Filament length (all spp.)	–0.200 (0.075)	–0.235 (0.019)	0.945
Filament length (bottle-brush spp. excluded)	–0.242 (0.021)	–0.124 (0.260)	
Number of flowers per inflorescence	—	—	0.742
Floral display (floral diameter × flowers per inflorescence)	—	—	0.895

Correlation coefficient increases ↑

Phylogenetic signal increases ↓

with long filaments and are part of the uncommon "small flower–large pollinator" syndrome (Willmer, 2011) (see Fig. 4).

Flower diameter was positively correlated (Pearson's correlation) with the three other characters chosen as possible flower size surrogates and highly significant ($P < 0.00001$, Fig. 5), showing that flower diameter has a strong relationship with all other flower size surrogates.

***PICs and phylogenetic signal results*—**Phylogenetic signal (lambda) for floral dimensions varied between 0.337 and 0.945 (Table 1). Characters with the lowest values of phylogenetic signal (i.e., less phylogenetically constrained characters) were also those that showed the strongest correlation with average number of flowers per inflorescence in an interesting inverse-ranked pattern. Pearson's correlation, that does not take phylogeny into account, showed that flower diameter (lowest phylogenetic signal, 0.337) had the highest correlation (–0.37) with average number of flowers per inflorescence. Characters with the highest values of phylogenetic signal (i.e., those with stronger phylogenetic inertia) showed the lowest correlations with average number of flowers per inflorescence. Slow-to-change filament length (highest phylogenetic signal, 0.945) also had the lowest levels of correlation (–0.20) with average number of flowers per inflorescence and was only marginally significant ($p = 0.055$), possibly due to the relation of this feature with breeding system (Willmer, 2011). Phylogenetic signal for average number of flowers per inflorescence itself (0.742) was intermediate, while the phylogenetic signal of floral display was higher than either flower diameter or number of flowers (0.895).

PICs results showed a similar pattern to Pearson's correlation for flower diameter, flower depth, and longest anther axis, with a slight reduction in all values, but maintaining their rank positions and significance except for filament length (Table 1). Filament length was an exception and showed a stronger and opposite response to phylogenetic correction: the correlation became higher and rose from marginally significant ($P = 0.055$) to significant ($P = 0.019$). It also rose two steps in rank, becoming the second most highly correlated character to flower number, although its phylogenetic inertia is very high. We reasoned that this behavior of the data might be due to our sample including four bottle-brush species; all of them are Myrtaceae and two are sister taxa (Appendix S3). Removing bottle-brush species from the analysis in Pearson's uncorrected correlation caused the correlation to become significant. When PICs were redone removing bottle-brush species, the correlation with flower number dropped back into its "correct" inverse rank position, but it also became nonsignificant ($P = 0.20$). Thus, the inverse rank pattern holds true for all species in Pearson's correlation, but when phylogenetic correction is applied, then it holds only if bottle-brush species are excluded.

DISCUSSION

If FDT-O is accepted as a recurrent evolutionary pattern in angiosperm evolution and energetic constraint as its driving

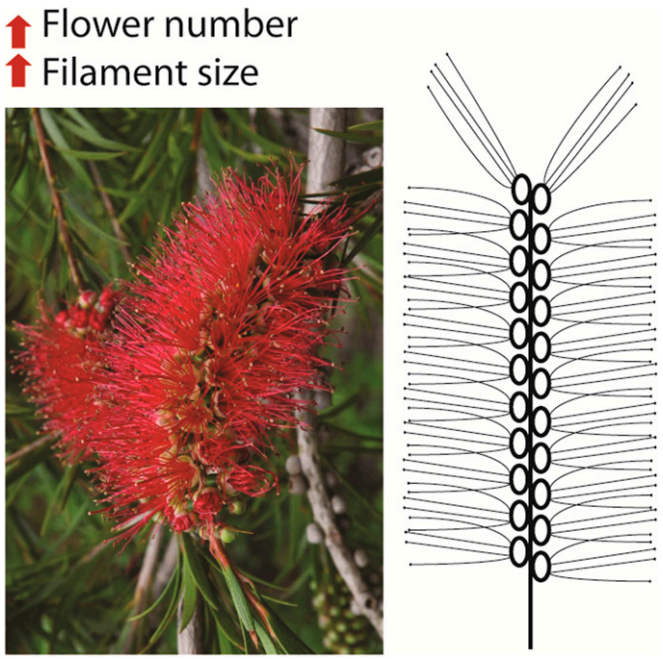

Fig. 4. *Callistemon* (Myrtaceae) presenting the bottle-brush strategy. Bottle-brush inflorescences represent an uncommon case of inflorescences with a simultaneously high investment on number of flowers and filament length, usually aimed at large animals. Image rights: Felix Forest.

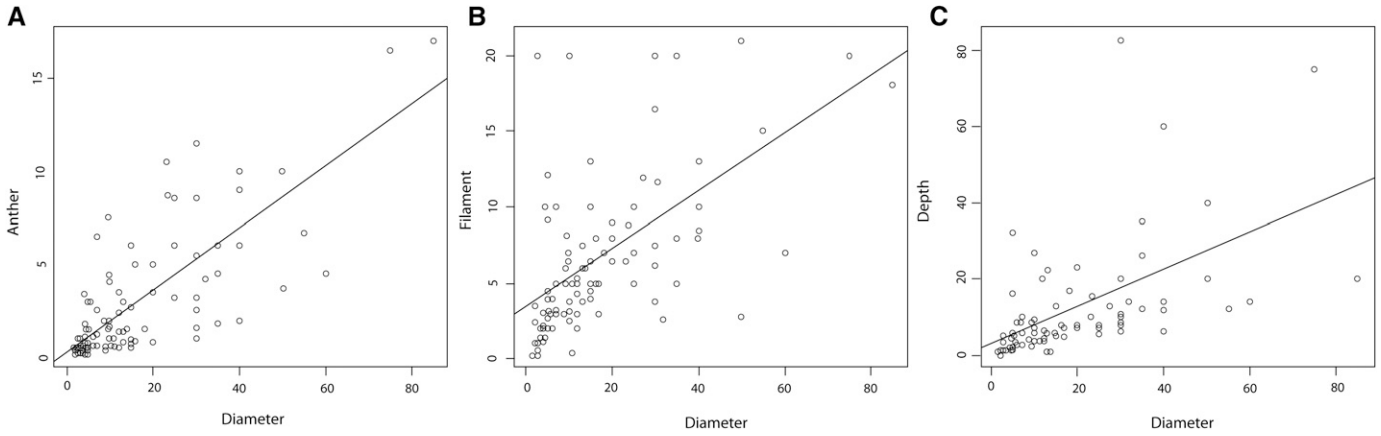

Fig. 5. Linear regression between flower diameter vs. (A) longest anther axis ($R^2 = 0.541$, $P < 0.00001$), (B) filament length ($R^2 = 0.346$, $P < 0.00001$), and (C) floral depth ($R^2 = 0.318$, $P < 0.00001$).

force, then a high negative correlation of a given character with the number of flowers per inflorescence may mean that this character requires a high investment in biomass for the flower to be functional. In the average Myrtalean flower (if bottle-brush flowers are not considered), biomass investment apparently increases in the following order: filament length < anther size < flower depth < flower diameter. It is noteworthy that flower depth, the second most significant energy sink among the chosen characters, has been shown to be a good predictor of nectar production when associated with number of flowers per inflorescence (Harder and Cruzan, 1990). Flower diameter has a very high correlation with other flower size surrogates, possibly because flower parts are often under similar selective pressures that make them increase or diminish their sizes simultaneously.

The shift in filament rank caused by including or excluding bottle-brush species shows the importance of considering adaptation to different pollinators in studies focusing on energetic constraint. Bottle-brush species presumably respond to a different set of selective pressures, as there is a shift in filament function from single flower pollen presentation to that of floral attractant and mass pollen presentation. Selection for many flowers with long filaments presumably breaks the trade-off "plus/minus" rule, i.e., the more flowers there are in an inflorescence the smaller their parts; bottle-brush flowers manifest a "plus/plus" selection: many flowers with long filaments that are also likely to be energetically cheaper attractants than petals. In the Myrtales, this syndrome is found in Myrtaceae in bird-pollinated *Callistemon* and *Metrosideros* (Carpenter, 1976) and bat-pollinated *Syzygium* and *Melaleuca* (Fleming et al., 2009) and in Combretaceae in bird-pollinated species of *Combretum* (Quirino and Machado, 2001). It is also found in some mimosoid legumes, such as in some species of *Parkia* (Hopkins, 1983).

Our results show that, in the Myrtales, characters with the highest presumed energetic costs (flower diameter, floral depth, and number of flowers per inflorescence) are also those with lowest phylogenetic signal, possibly because a more labile character will respond more quickly to selective pressures to increase or decrease floral size. Changes in environmental pollinator availability are believed to have led to changes in energetic allocation in different floral parts along the evolutionary history of several groups of angiosperms (Proctor et al., 1996; Weller et al., 2006). Shifts in the relative size of floral size characters (and consequently in flower number, due to FTD-O) are sometimes associated with shifts in pollinator class, even among phylogenetically close congeners. This might weaken correlations between flower size characters but apparently not enough to undermine the broad-scale pattern across the order. In our sample, examples of such shifts are recorded in the Lythraceae and the Onagraceae. In the Lythraceae, *Cuphea llavea* and *C. hyssopifolia* have flowers with deep floral tubes, but while the diameter of the corolla doubles between *C. hyssopifolia* and *C. llavea*, floral depth increases by a factor of four, and is possibly an adaptation to *C. llavea* being hummingbird-pollinated (Bortolameotti, 1981; Graham, 1994; Kubitziki, 2006; Gonzalés, 2009). In the Onagraceae, *Fuchsia cyrtandroides* has a single, large, solitary flower (ca. 30 mm diameter, Wagner et al., 2007), while *F. paniculata* has an inflorescence with 80+ flowers that are ca. 7 mm in diameter (Wagner et al., 2007). Although we are not aware of any reproductive biology studies of these two species of *Fuchsia*, their floral syndromes suggest that *F. cyrtandroides* (pendulous flowers with thick, deep crimson corollas and an exserted, contrasting yellow-green stigma) is likely to be bird-pollinated, while *F. paniculata* (upright flowers with thin, pink corollas) is likely to be bee-pollinated.

The fact that the floral display has a higher phylogenetic signal than either flower size or the number of flowers per inflorescence individually shows that the total energetic cost of the floral display is strongly phylogenetically constrained. If both flower diameter and flower number are more adaptable and quicker to change, then lineages can modify their flowering strategies without having to change the total cost of inflorescence production.

***Conclusion*—**Flower diameter, intuitively chosen by biologists and ecologists as a floral size variable, does indeed seem to be the best single estimate of individual floral cost. Studies using this variable to estimate "flower size" are also likely to simultaneously take into account other floral size variables because of the high level of correlation among them, especially when a variety of flower strategies is considered in the sample.

An unforseen possible pattern that merits further investigation is that the more a floral character costs, the less phylogenetically constrained it can afford to be. Additional floral cost variables (e.g., flower color, bract dimension, nectar volume)

should be included to test whether a larger sample size (as yet insufficient) will result in a significant negative correlation between floral cost and phylogenetic signal. We furthermore suggest that higher taxa fitness and adaptability, at least as far as floral display is concerned, may depend upon an ability to maintain adaptability of floral dimensions and number of flowers per inflorescence, while the overall investment in floral display is more static over evolutionary time. Similar investigations in other large, ancient orders, such as Malpighiales, Malvales, and Fabales may show that this is a particular characteristic of megadiverse higher taxa.

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APPENDIX 1. GenBank accession number for all molecular information used in the phylogenetic analysis.

Taxon, GenBank accession: *rbcL*, *ndhF*

***Alzatea verticillata* Ruiz & Pav.,** U26316, AF215591; ***Bucida macrostachya* Standl.,** U26321, AY498839; ***Conocarpus erectus* L.,** AF281477, AY498840; ***Quisqualis indica* L.**, L01948, AY498841; ***Terminalia catappa* L.,** U26338, AY498842; ***Axinandra zeylanica* Thwaites,** AY078157.1, AJ605094.1; ***Crypteronia griffithii* C.B.Clarke,** AJ605087.1, AJ605098.1; ***Crypteronia paniculata* Blume**, AY078153, AJ605099.1; ***Dactylocladus stenostachys* Oliv.**, AY078156.1, AJ605100.1; ***Cuphea hyssopifolia* Kunth**, AM235672.1, AM235439.1; ***Cuphea llavea* Lex.**, AF495773, AF495796; ***Duabanga moluccana* Blume**, AY496862; AY498835, ***Lawsonia inermis* L.**, AY496863, AY498836; ***Lythrum salicaria* L.,** AF495760, AF495775; ***Nesaea aspera* (Guill. & Perr.) Koehne,** AY496864, AY498838; ***Trapa natans* L.**, L10226, AY498838; ***Amphiblemma cymosum* Naudin,** AF215543, AF215588; ***Arthrostemma ciliatum* Pav. ex D. Don**, AF215522, AF215562; ***Astronia macrophylla* Blume,** AF215510, AF215548; ***Astronia smilacifolia* Triana,** AF215511, AF215549; ***Bertolonia maculata* DC.,** AF215512, AF215550; ***Blakea trinervia* L.,** AF215516, AF215555; ***Calvoa orientalis* Taub.,** AF215544, AF215589; ***Clidemia rubra* (Aubl.) Mart.,** AF215535, AF215579; ***Dichaetanthera asperrima* Cogn.,** AF215523, AF215564; ***Diplectria divaricata* Kuntze,** AF270746, AF215556; ***Graffenrieda rotundifolia* (Bonpl.) DC.,** AF215532, AF215576; ***Heterocentron subtriplinervium* (Link & Otto) A. Braun & C.D. Bouché,** AF270747, AF215566; ***Heterotis rotundifolia* (Sm.) Jacq.-Fél.,** U26323, AF215565; ***Lavoisiera cordata* Cogn. ex Glaz.,** AF215540, AF215582; ***Leandra mexicana* (Naudin) Cogn.,** AF215536, AF215580; ***Macrocentrum repens* (Gleason) Wurdack,** AF215513, AF324498; ***Maieta guianensis* Aubl.**, AF215537, AF215581; ***Medinilla humbertiana* H. Perrier,** AF215517, AF215557; ***Melastoma malabathricum* L.,** AF270748, AF272810; ***Memecylon edule* Roxb.,** AF215515, AF215574; ***Meriania nobilis* Triana,** AF215533, AF215577; ***Miconia calycina* Cogn.,** AM235650.1, JF832003.1; ***Miconia dodecandra* Cogn.,** EU711396.1, EU056026.1; ***Miconia pyramidalis* (Desr.) DC.,** JF832004.1, EU056080.1; ***Microlicia fasciculata* Mart. ex Naud.,** AF215541, AF215583; ***Monochaetum calcaratum* (DC.) Triana,** AF215524, AF215568; ***Monolena primuliflora* Hook. f.,** AF215514, AF215553; ***Mouriri guianensis* Aubl.,** AF215529.2, AF215575; ***Osbeckia stellata* Buch.-Ham. ex Ker Gawl.,** U26330, AF272818; ***Pternandra caerulescens* Jack,** AF215518, AF215558; ***Rhexia virginica* L.,** U26334, AF215587; ***Rhynchanthera grandiflora* (Aubl.) DC.,** AF215542, AF215584; ***Tetrazygiopsis urbanii* (Cogn.) Borhidi,** AF215538, AF270753; ***Tibouchina urvilleana* (DC.) Cogn.,** U26339, AF272820; ***Tococa guianensis* Aubl.,** AM235650.1, AY498834.1; ***Triolena obliqua* (Triana) Wurdack,** AF215518, AF215558; ***Angophora hispida* (Sm.) Blaxell,** U26317, AY498763; ***Backhousia citriodora* F.Muell.,** U26318, AY498768; ***Callistemon citrinus* (Curtis) Skeels,** AM235652.1, AM235419.1; ***Eucalyptus globulus* Labill.,** HM849985.1, AY780259.1; ***Eugenia uniflora* L.,** AF294255, AF215592.1; ***Euryomyrtus ramosissima* (A.Cunn.) Trudgen,** U26319, AY498782; ***Heteropyxis natalensis* Harv.,** U26326, AY498824; ***Kunzea ericifolia* (Sm.) Heynh.,** AM235655.1, AM235422.1; ***Leptospermum scoparium* J.R.Forst. & G.Forst.,** AM235656.1, AM235423.1; ***Lophostemon confertus* (R.Br.) Peter G.Wilson & J.T.Waterh.,** AM235657.1, AY498794.1; ***Melaleuca quinquenervia*(Cav.) S.T.Blake,** GU135164.1, EU410162.1; ***Metrosideros nervulosa* C.Moore & F.Muell.,** AJ235785, AY498802; ***Myrcianthes fragrans* (Sw.) McVaugh,** U26328, AY498803; ***Myrtus communis* L.,** AF294254, AF215593; ***Psidium cattleianum* Afzel. ex Sabine,** HM850290.1, HM160101.1; ***Psiloxylon mauritium* (Bouton ex Hook.f.) Baill.,** U26333, AY498825; ***Syzygium smithii* (Poir.) Nied.,** U26315, AY498760; ***Uromyrtus metrosideros* (F.M.Bailey) A.J.Scott,** AM235661.1, AM235428.1; ***Calylophus hartwegii* (Benth.) P.H.Raven,** AF495767, AF495790; ***Circaea alpina* L.,** L10216, AF495780; ***Clarkia xantiana* A.Gray,** L10225, AF495787; ***Epilobium angustifolium* L.,** L10217, AF495784; ***Epilobium rigidum* Hausskn.,** AF495763, AF495785; ***Eremothera boothi* (Douglas) W.L.Wagner & Hoch,** AF495766, AF495790; ***Fuchsia cyrtandroides* J.W.Moore,** L10220, AF495779; ***Fuchsia paniculata* Lindl.,** AM235667.1, AM235434.1; ***Gaura mutabilis* Cav.,** AF495769, AF495792; ***Gayophytum heterozygum* F.H.Lewis & Szweyk**., AF495765, AF495788; ***Gongylocarpus fruticulosus* (Benth.) Brandegee,** AF495762, AF495783; ***Hauya elegans* DC.,** L10227, AF495778; ***Lopezia riesenbachia* Plitmann, P.H.Raven & Breedlove,** L10219, AF495781; ***Ludwigia peploides* (Kunth) P.H.Raven,** L10222.1, AF495776.1; ***Ludwigia peruviana* (L.) H.Hara,** L10221, AF495777; ***Megacorax gracielana* M. González & W.L. Wagner,** AF495774, AF495797; ***Oenothera elata* Kunth,** AF495771.1, AF495794.1; ***Stenosiphon linifolius* (Nutt. ex E. James) Heynh.,** AF495768, AF495791; ***Xylonagra arborea* (Kellogg) Donn. Sm. & Rose,** AF495764, AF495786; ***Brachysiphon acutus* (Thunb.) A.Juss.,** AJ605084.1, AJ605095.1; ***Endonema retzioides* Sond.,** AJ605088.1, AJ605101.1; ***Olinia emarginata* Burtt Davy,** AJ605089.1, AJ605102.1; ***Olinia ventosa* (L.) Cufod.,** AF215546, AF215594; ***Penaea mucronata* L.,** AY078155, AF270756; ***Rhynchocalyx lawsonioides* Oliv.,** U26336, AF270757; ***Saltera sarcocolla* Bullock,** AJ605091.1, AJ605103.1; ***Erisma floribundum* Rudge,** U26324, AY498827; ***Qualea albiflora* Warm.,** JQ626202.1, AM235431.1; ***Vochysia guatemalensis* Donn. Sm.,** U26340, AY498832.